

Regulation of Immune Functions in the Fetus and Newborn¹

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I. Introduction

The timing of maturation of immunologic responses varies considerably in mammals with the capacity to respond to antigen appearing in utero for some species while in others immunocompetence is achieved only after birth

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[450]. Limited expressions of immune responsiveness can occur, for example, very early in fetal life as in the sheep, cow, monkey, and man, or not until well into the postnatal period as is the case for the common laboratory rodents [430]. However, it is generally agreed that the developing fetus and newborn remain throughout the prenatal, and much of the neonatal period relatively immunoincompetent in comparison to the adult. The process of pre- and postnatal development from immunological immaturity to full immunocompetence appears to represent a well-ordered sequence of stepwise events leading to the gradual establishment of competence [430, 450]. Furthermore, a hierarchy of antigens has been shown to exist to which the developing young of a given species displays a characteristic capacity to respond at precise maturational stages [424, 430]. The position of an antigen in this hierarchy remains constant for the individuals within a particular inbred strain of mice, but varies between different strains suggesting a role for certain genetic factors in the acquisition of immune responsiveness during ontogeny [425]. Results from a number of different studies indicate that the orderly appearance of immunocompetence during ontogeny is not directly associated with the development of antigen-specific receptors on lymphocytes. Thus, there is good evidence that the repertoire of antigen-binding cells in adult and young animals is similar [119, 234, 431], and that these cells arise early in development at a time which may precede the ability of the animal to mount a specific immune response [108, 111].

It is now becoming increasingly clear that humoral and cell-mediated immune responses to most antigens are the result of a complex series of well-balanced macrophage-dependent T-T cell, and T-B cell interactions. Immunological homeostasis is maintained by complicated networks of feedback inhibitory cell circuits [69] in which macrophages would seem to serve to transmit T cell-derived communication signals to other T cells within a circuit [378]. The relative lack of responsiveness during ontogeny could thus be due to functional failures at one or more of the cellular interactions which occur in a normal immune response. An attempt will be made in the present article to relate what is presently known about immune deficiencies in the fetus and newborn to current concepts of induction and regulation of humoral and cell-mediated immunity.

While the immunological immaturity of the fetus and newborn does extend to both humoral [369] and cell-mediated reactions [202], ontogenetic studies frequently fail to reveal any obvious quantitative deficiency in the cellular constituents considered necessary to initiate an immune response [443]. These findings do not preclude, however, the likely necessity for further

qualitative changes in the lymphoid cell populations subsequent to the appearance of recognizable B and T cells with their apparent full range of antigen-binding specificities. It is notable in this regard that B lymphocytes undergo well-defined phenotypic shifts in their cell surface markers, including immunoglobulin, antigens coded by the Ia immune response genes and the complement C3 receptor [180, 181], as well as Lyb 3 [205], Lyb 5 [11] and MLs [12] determinants. Analogous time-dependent maturational events may have to occur in the antigen-presenting mechanism mediated by subpopulations of macrophages [105, 330, 331], and in functionally distinct subsets of T cells such as those defined by Ia [133] and by Lyt antigens [70, 133].

It is clear that both quantitative and qualitative changes in the lymphoid cell populations contribute to the emerging pattern of immune responsiveness during early ontogeny. However, there is now also a considerable amount of evidence that the developing young has an inherent capacity to respond to antigen much earlier than previously suspected, but that this potential for immune reactivity may be masked by dominating immunosuppressor mechanisms. One of the first indications for this come from a study [113] in which it was demonstrated that sensitized adult rabbit lymphoid cells give a relatively poor immune response when transferred to neonatal recipients whereas such cells were capable of strong antibody responses when transferred to irradiated adult recipients. Conversely, it was also shown that immune cells from neonatal rabbits did not function optimally in neonatal recipients, but they did when transferred to irradiated adult recipients [114]. The authors concluded that the immunological inadequacy of the neonate is probably related more to its inhospitable internal environment than to a lack of cells capable of antibody formation. More recently it has been shown that adoptive transfer of neonatal spleen cells to adult, lethally irradiated carrier-primed recipients maximizes the responsiveness of neonatal B cells [374]. This and other reports [279] indicate that while neonatal and adult cells demonstrate some quantitative differences in their responses, the relative immunodeficiency in newborn animals may not be primarily due to an inadequate population of responding B cells. Several other studies also suggest that the poor immune response of neonatal animals cannot be attributed solely to an underdeveloped antigen-processing system [138, 271, 379]. These findings together with the observation that newborn mice do not lack T helper cell function [379] lead one to conclude that neonatal incompetence is, at least in part, a manifestation of an overriding active humoral and/or cellular suppression.

It is well recognized that the maternal environment can exert consider-

able influence on the developing immune systems in the fetus and newborn [208, 263, 272, 438]. This article will therefore also be concerned with some of the immunoregulatory aspects of the fetal/maternal relationship. The mammalian fetus can, according to the extensive studies of *Beer and Billingham* [38] be considered as an allograft at all stages of its existence. The total lack of maternal/fetal vascular communication as well as the presence of an effective neutral barrier (the syncytiotrophoblast) serve to distinguish the fetal allograft from conventional graft-host relationships [37]. However, the anatomical separation of maternal and fetal environment is incomplete and there is considerable bidirectional transplacental traffic of both humoral and cellular components [126]. The mutual sensitization which results from this exchange of antigenic material during gestation is thought to have both potentially harmful and beneficial effects on maintenance of the fetal allograft [39]. While it is now clear that several factors must be contributing to the success of the fetal allograft, there is much evidence to suggest that one important factor is a selective depression of the maternal immune system representing an adaptive response to protect the fetus from premature rejection [40].

The purpose of this article is (a) to describe the functional development of various cell types in the immune system during ontogeny, and (b) to examine the impact of naturally occurring maternal/fetal-newborn immunoregulatory forces on the emerging immune-response patterns. The literature cited has therefore been selected with this goal in mind and no attempt has been made to review comprehensively the immunobiology of the mammalian fetus and newborn. This review will concentrate on data obtained primarily in murine and to a lesser extent human systems since many of the relevant studies have been carried out in these species.

II. Ontogeny of T Cell Functions

A. Thymus

Thymus-derived T lymphocytes are involved, directly as effectors or indirectly as positive and negative regulators, in most types of specific immune reactions. T cells originate from fetal yolk sac, liver, or adult bone marrow-derived hematopoietic stem cells which migrate to the thymus via the bloodstream [351, 460]. Initial differentiation events within murine thymus involve the conversion of stem cells to thymocytes which are corticosteroid sensitive [49, 50], relatively immunoincompetent [50, 99], and bear

high density of Thy-1 [392] and TL [340] cell-surface antigens. Thy-1 and TL surface antigens have served as convenient markers to follow T cell development from immature cortical thymocytes to the more mature medullary T cells. The maturational process which occurs as thymocytes move from the cortex to the medulla [52, 411] results in the loss of TL antigens, a reduction in the amount of Thy-1 antigen, and the acquisition of immunocompetence. In general, medullary thymocytes resemble peripheral T cells with respect to Thy-1 density and relative resistance to irradiation and to corticosteroid treatment. Recent studies have shown that it is possible to compartmentalize the remarkably heterogeneous T cell populations into functionally distinct subsets on the basis of mitogen responsiveness [292, 455], rosette formation [412], Fc receptors [293, 457], the quantity of Thy-1 antigen [73], as well as cell surface Lyt [70, 198], Ia [57, 58, 211, 323, 466], and Qa-1 [445] antigen phenotypes. Many of these T lymphocyte subsets have stable cell-surface antigen phenotypes and are programmed for distinct functions [69]. It is clear that the ultimate manifestation of a T cell-dependent immune response is the result of a complex series of T-T synergistic [71, 72] and antagonistic [196, 395] interactions in which macrophages play an important intermediary role [378]. If one or more of the various effector and regulatory T cell subsets are out of normal physiological balance then an abnormal immune response is to be expected. Indeed, this has already been shown to be the case with the autoimmune disease-prone mouse strain NZB which has a defective Lyt 123⁺ T cell subset [74], and in MRL mice which have a malfunction in the Lyt 1⁺ subset [153].

The murine fetal thymus appears to contain thymocyte precursor cells as early as day 12-13 of gestation since these cells are capable of becoming lymphoid in organ culture [81]. The number of lymphoid cell precursors in the thymus at day 13 ranges from 7,000 to 15,000, and these cell numbers increase exponentially thereafter to reach a plateau by 18-19 days of gestation [216]. By day 19 of gestation most fetal thymocytes express approximately adult levels of Thy-1, Lyt 1⁺, and Lyt 2⁺ antigens [216]. While the peripheral T cells of neonatal mice are thought to be predominantly of the Lyt 12⁺ phenotype [70] the cells which migrate out of the thymus of newborn mice are 60-70% Lyt 1⁺ and 30% Lyt 12⁺ [419]. A similar proportion of Lyt subsets has been reported for adult thymus migrants [419, 420] and for adult cortisone-resistant thymocytes [269, 419]. The neonatal thymocyte population has also been characterized as having a reduced number of small and medium size cells and an enhanced proportion of large, blast-like cells relative to the distribution of these cell types in the adult [325].

Mosier [300] and *Mosier and Cohen* [301], have studied the responsiveness of mouse thymocytes from day 16 of gestation through adulthood to various mitogens and alloantigens. Fetal thymocytes were found to be transiently more reactive in the MLR and to PHA than at any other time in the life of the animal. Reactivity to Con A and PWM increases gradually during the late fetal and early neonatal period and reaches adult levels between 2 and 3 weeks of age. Interestingly, the ability of neonatal thymocytes to respond to allogeneic cells in the MLR diminishes during the first 2 weeks of life but then returns to adult levels [300]. Additional studies by *Cohen and Mosier* [101] showed that hydrocortisone-resistant newborn thymocytes, which represents about 15% of the normal untreated newborn thymus, could account for all the PHA, Con A, and PWM reactive cells. In contrast, another recent report [397] finds that Con A responsiveness can first be demonstrated in murine fetal thymus at 18 days of gestation followed by the appearance of PWM reactivity at birth. In this study Con A reactivity rose slowly to reach a maximum after 2 weeks of age while PWM responses reached a peak within 1-2 days after birth. PHA responses remained low or negligible throughout the first 15 days of the post-natal period. The peak of PHA reactivity before birth as described elsewhere [323, 467] was not observed in this study. The weak or absent reactivity of newborn thymocytes to PHA [8, 71, 202] was reported to remain unchanged even after the administration of cortisone acetate to cell donors [202], a treatment which dramatically enhances adult thymic responses to PHA. In another comprehensive study of mitogen reactivity in the developing murine thymus *Byrd et al.* [63] also showed that newborn mice do not respond to PHA, but responded well to PWM. Reactivity to PHA was shown to occur later during young adulthood and reached a plateau by 8-12 weeks of postnatal life [202]. *Stobo and Paul* [456] showed that although the frequency of Thy-1-positive cells in newborn mouse thymus was equivalent to that of adult thymus, newborn thymocytes possessed very little reactivity to Con A. Thymocytes were shown to acquire Con A reactivity with age reaching a maximum at 4 weeks without either a change in the average number of cell-surface theta determinants, or any significant increase in the frequency of Thy-1-positive cells [456].

The appearance of MLR reactivity in rodent thymocytes is a very early pre- [323, 467] or postnatal [235] event, but the ability of T cells from the thymus to generate significant CML could not be detected until 1 week after birth [304]. However, while CML activity generated by neonatal thymocytes is weak, the lytic capacity per cell was found to be similar to that of adult lymphocytes. Studies on the ontogeny of graft versus host reactivity showed

that thymocytes developed the capacity to mount a GVH as measured in lethally irradiated adult recipients in the first few hours after birth [168]. The magnitude of the GVH reactivity by newborn thymocytes did not increase with age, as cells from 60-day-old animals had the same activity as similar numbers of cells from 3-day-old mice [168]. *Chakravarty et al.* [81] obtained similar results employing an in vitro GVH assay. They were unable to obtain GVH reactions with thymocytes taken at day 19 of gestation, but found that newborn thymocytes were as effective as adult cells in inducing splenomegaly in vitro [81]. *Chiscon and Gohub* [88] studied the ontogeny of T helper cell functions in the thymus by determining the age at which thymocytes acquire the ability to collaborate with adult B cells in lethally irradiated recipients to produce an antibody response to SRBC. They found that this function remained less than 10% of adult values until birth. At birth T helper cell function increased exponentially, reaching adult levels within 48 h [88]. *Chakravarty et al.* [81] have also shown that day-16 embryonic thymocytes possess the capacity to collaborate with adult B in vitro to produce anti-SRBC antibody if the T cells were appropriately activated by 24 h of preincubation with Con A. In this system while the activity of day-16 embryonic thymus was low, the mitogen-activated thymic helper activity of day-18 embryos was identical to adult thymus [81].

The ontogeny of intrinsic immunocompetence in the thymus would, on the basis of the findings summarized above, seem to coincide approximately with the morphological appearance of lymphocytes. It is important to note, however, that these conclusions are based to a large extent on functional tests of developing lymphocytes in culture or in adult environments following adoptive transfer. The degree to which this reactivity is actually expressed within the physiology of the fetus and newborn will be discussed below.

B. Spleen

T lymphocytes found in characteristic thymus-dependent locations in peripheral lymphoid tissue are believed to largely originate from the small population of medullary thymocytes which account for most, if not all of the immunocompetence within the thymus [49, 50, 99, 101, 110, 254]. The ontogeny of thymus-derived T cell functions has been extensively studied in the murine spleen. *Spear et al.* [443] have determined the percentages of Thy-1-positive spleen cells as a function of age employing an immunofluorescence technique. T cells could be detected in the spleen as early as day 15 of gestation, and by day 16, 8% of the total nucleated cells were Thy-1-positive. Numbers of splenic T cells then increased rapidly until ap-

proximately 1 week after birth at which time they represented more than 75% of normal adult values. Other studies employing a cytotoxic assay based on chromium release [456] or immunofluorescence localization of T cells using rabbit antimouse thymocyte antibodies [145, 492] have reported considerably lower numbers of splenic T cells at birth. However, the high percentage of Thy-1+ cells found in blood, lymph nodes, and Peyer's patches at birth [82, 388] may support the claim that newborn mice possess a nearly adult frequency of T lymphocytes in the spleen [443]. There is very little increase in T cell numbers between 1 and 2 weeks of age. Nevertheless, it is during this period that functional capacity of spleen cell populations begins to emerge in terms of mitogen [63, 202, 302, 442, 456] and alloantigen [9, 168, 202, 302] induced reactivity. Newborn spleen cell responsiveness to PWM was found to be substantial at 3 days of age, while significant reactivity to PHA was lacking until 17 days [202]. In this study both MLR and PWM reactivity was detectable in the spleen before PHA responsiveness. The refractoriness of neonatal spleen cells to PHA could not be overcome by varying the number of cells or the concentration of PHA. Kinetic studies by *Spear and Edelman* [442] showed that the number of spleen cells responding to Con A rose sharply from birth to 3 weeks of age, while PHA-reactive cells were not detected until 3 weeks of age and did not reach adult levels until 8 weeks. *Stobo and Paul* [456] reported that spleen cells from neonatal mice are virtually unreactive to Con A and PHA. They also demonstrated that spleen cells from 2-, 3- and 4-week-old animals came closer to the adult level of Con A responsiveness than of PHA responsiveness.

The response of spleen cells from mouse strains C57BL/6, CBA, and A/J to alloantigenic stimulation in MLR has been studied at closely spaced intervals from birth to 56 weeks of age [9]. No reactivity to alloantigens was present at birth. Following an unresponsive period lasting from 1 to 4 weeks depending on the strain combination, there was a sharp linear increase in alloreactivity which peaked at 14-20 weeks of age. However, in line with thymocyte data [235, 300, 301, 304] it was recently shown that spleen cells from newborn B10 mice are capable of responding to allogeneic stimulation by B10.D2 in primary MLC, whereas CML activity as measured by killing of PHA-stimulated B10.D2 target cells was not detectable until day 7 [498]. Poor CML by spleen cells from 4- to 5-day-old B10 mice could not be improved by varying the ratio of responding to stimulating cells indicating that the dichotomy of MLC and CML ontogeny was unlikely to be due to suboptimal culture conditions. Moreover, the ontogenetic sequence was not simply a reflection of a gradual increase in the magnitude of the MLC phase

of the CML. Thus, the MLC response was not greater in 13- to 16-day-old than 4- to 5-day-old animals, yet the older mice demonstrated excellent CML while in the younger animals no CML was observed. *Pilarski* [367] subsequently developed a very efficient method for generating cytotoxic T cells which allowed the demonstration that the ability of spleen cells to generate CML in vitro had in fact begun to develop by 3 days after birth in Balb/c mice. By comparison, CBA spleen required 8-9 days of postnatal development before cytotoxicity levels reached the day-4 level of Balb/c mice. Addition of antigen-specific helper cells, considered to be required for [368] or at least helpful to [27, 100] the effective generation of CTL, did not augment CTL development in newborn mice. A lack of adherent/accessory cell activity in the newborn was shown not to be a limiting factor in these experiments [367]. The Lyt phenotype of alloreactive prekiller cells in the spleen of donors at increasing intervals after birth have been determined [61]. Spleen cells from 2-week-old B6 mice were shown to generate Lyt 23⁺ cytolytic effector cells after stimulation with DBA/2 spleen cells. Lyt 123⁺ cells are required for generation of Lyt 23⁺ killer cells indicating that the phenotype of alloreactive prekiller cells at 1 week after birth could be Lyt 123⁺. Examination of the Lyt phenotype of prekiller cells in progressively older mice revealed that at approximately 3-5 weeks of age the prekiller activity shifts from the Lyt 123⁺ compartment to the Lyt 23⁺ compartment [61].

CBA spleen cells from mice younger than 3 days of age were shown to be unable to elicit a significant GVH response in B6AF₁ mice [168]. In this study GVH reactivity was first evident at approximately day 4, and the response with a given number of transferred cells increased rapidly with increasing age of donor mice reaching adult levels within 14 days. In a separate study GVH reactivity was reported to be present in murine spleens after day 1 with full competence achieved as early as 4 days after birth [479].

Helper T cell function for T-dependent antibody synthesis does not reach adult-like activity until 4-5 weeks of age [443, 470]. Antibody responses to T-dependent antigens have been shown to occur earlier and with increased magnitude when helper T cell function is substituted or enhanced by mitogen [215, 329, 333, 414, 442] or thymus extract [53, 183]. The ontogenetic maturation of splenic T cells has also been followed by the ability to produce a T-cell replacing factor (TRF) which can restore T-dependent antibody responses in an in vitro T-cell deficient system [53]. Cultures of nude spleen cells could be stimulated to produce anti-SRBC antibodies in the presence of TRF obtained from adult cells but not from neonatal spleen cells. Only

after the mice were 1 to 2 weeks of age did their spleens have T lymphocytes which produced TRF [101].

Studies on the ontogeny of murine splenic T cells and their functions have been largely restricted to the neonatal period. This is a time when newborn animals will be presented with an array of foreign substances and infectious microorganisms. This may in part explain the sometimes quite different findings in the various functional assays in terms of the time of appearance of a particular immune reaction. We would conclude that very low numbers of T cells are present at birth in the spleen, but that they then rapidly increase to adult levels. Careful studies have also revealed that the neonatal murine spleen would seem to contain T cells of all known subsets but with different proportional distributions from adult spleen.

III. Ontogeny of B Cell Functions

B lymphocytes are derived from pluripotential hematopoietic stem cells [125, 497] which are themselves originally the progeny of primitive cells from the embryonic yolk sac [290]. Analysis of murine B cell development has indicated that the fetal liver is most likely the major site of cell differentiation in the embryo [365]. B lymphocytes normally develop multifocally in liver, spleen and bone marrow beginning about 3 days before birth [334, 352, 389]. Within a few days after birth the liver stops generating B cells [280, 403], and it is generally assumed that the differentiation of stem cells to B cells then shifts to the bone marrow [352]. Cells at the earliest recognizable stage in the B lymphocyte lineage are called pre-B cells. Such cells have cytoplasmic IgM, but no cell surface immunoglobulin detectable by immunofluorescence [244]. The actual time of appearance of murine pre-B and B cells varies somewhat from study to study depending on the particular definition of 'B cells', the method used to detect them, and the strains of mice tested. Employing immunofluorescent techniques Raff et al. [389] have described pre-B cells in murine fetal liver at 12 days gestation, approximately 5 days prior to the appearance of liver cells with detectable IgM on their surface [353]. Melchers et al. [281] have used sensitive biosynthetic labeling to demonstrate 7-8S IgM synthesis in fetal liver cells as early as day 11 of gestation, and the presence of surface-bound IgM on liver cells between days 12 and 15. B cells in the fetal liver [281, 403] and spleen [403] become mitogen reactive at birth, and by 7 days of age functional B cells in the spleen have reached 1/3 the adult level [403]. At about the same

time that fetal liver B cells become reactive to mitogen, they also become inhibitable by anti-immunoglobulin antibodies [281]. Newly developed IgM surface positive B cells in the fetus and newborn can be distinguished from their adult counterparts on the basis of sensitivity to anti-immunoglobulin-induced suppression [245, 264, 317]. Thus, *Raff et al.* [390] have shown that treatment with anti-IgM antibodies in vitro may induce irreversible modulation leading to permanent functional inactivation of immature B lymphocytes, while similar treatment of adult B cells has a reversible impact only. Antibodies synthesized by fetal and neonatal B cells have been shown in cell transfer studies using T-dependent antigens to be highly restricted with respect to their heterogeneity of avidity of the plaque-forming cells (PFC) compared with the PFC response of adult B cells [165]. Murine B cells acquire the capacity to produce an adult-like heterogeneous antibody response between 7 and 10 days of age. It was subsequently shown using another approach that as early as 14 days of gestation, B cell precursors in fact possess the genetic information required to synthesize an adult-like heterogeneous response [166]. The authors suggested that a differentiation event occurs between 7 and 10 days of age allowing the expression of a heterogeneous B cell response [166]; however, the contribution of an immunoregulatory process has not been ruled out. It has been shown by direct immunization of intact animals that young mice synthesize only IgM antibodies followed several days later by the capacity to synthesize significant amounts of IgG antibodies [80, 471]. However, cell transfer studies using syngeneic adult irradiated recipients supplied with an excess of adult T cells have shown that B cell precursors already have acquired the potential ability to produce IgG antibody by day 14–16 of fetal life [166].

The reason(s) for the poor antibody responsiveness of neonatal animals to conventional thymus-dependent antigens [138, 442, 443] cannot be attributed to any gross deficiency in numbers of Thy-1-bearing T cells and immunoglobulin-bearing B cells [443]. Thus, *Spear and Edelman* [442] and *Spear et al.* [443] noted that in spite of the prenatal appearance of sizable numbers of recognizable B cells and T cells in murine spleen, no detectable level of humoral antibodies occurs until after 1 week of age, and adult-like responsiveness is not achieved until 4–8 weeks of age. Moreover, several studies have clearly shown that the ability of newborn animals to respond to T cell-independent antigens precedes the capacity to produce antibodies to T cell-dependent antigens during ontogeny [186, 301, 305, 387]. The diminished capacity of the newborn to mount antibody responses may thus be partly due to a requirement for subtle postnatal maturational events result-

ing in changes in B cell functional properties. Indeed, it was earlier proposed [151, 370] and subsequently shown that several distinct B cell subsets exist which express individual response patterns to antigens which can be defined on the basis of cell-surface marker phenotypes [173, 214, 256, 257, 375]. It is possible to link these findings with ontogenetic studies which indicate that B lymphocytes proceed through several distinct maturational stages characterized by the expression of unique surface marker phenotypes at each stage [149, 182, 222, 417, 427, 485, 486]. For example, in the mouse from about 14 days of gestation until 7-3 days after birth, IgM is the only readily detectable isotypic marker on B cells [222, 417, 485, 487]. After 1 week of age a separate population of small splenic lymphocytes bearing both IgM and IgD [2, 170] appears and gradually increases until by 3 weeks of age 60% of spleen B cells exhibit this phenotype [222, 485]. As young adulthood is approached, at least three distinct populations of B cells exist in murine lymphoid tissues, namely cells bearing only IgM, those bearing both IgM and IgD, and cells bearing only IgD [486]. Mosier et al. [306, 307] have studied the functional consequences of the switch of B cell populations bearing primarily IgM to those bearing primarily IgD during ontogeny. It was noted that one group of T cell-independent (TI) antigens (i.e. the so-called TI type 1 antigens, which includes TNP-*Brucella abortus*) can effectively induce newborn spleen cells to produce specific antibodies in vitro. However, a second group of T-independent antigens (TI type 2, including TNP-Ficoll) was found to require a more advanced stage of B cell differentiation before antibodies could be induced. It was determined that the triggering process for responsiveness to TI 2 antigens requires interaction with an IgD⁺ B cell subset. The authors concluded from these findings that the acquisition of IgD is essential to the increase in immunocompetence expressed in developing young mice. In addition, other studies suggest that the phenotypic conversion of Ia⁻ B cells to Ia⁺ B cells is relevant to the changing response patterns during postnatal development [182]. Lewis et al. [257] have clearly shown that the expression of Ia antigens defines two subpopulations of adult B lymphocytes. The Ia⁻ primary B cell subpopulation contains precursors which give rise to only IgM antibody production, whereas the Ia⁺ subpopulation contains cells capable of also giving rise to IgG antibody synthesis. At birth IgM⁺ murine B lymphocytes are essentially all Ia⁻ [182, 222]. After birth, however, there is a linear increase in the percentage of IgM⁺ cells which express Ia, so that by 9 days of age 95% of splenic IgM⁺ cells are also Ia⁺ as is the case for adult spleen cells [222]. It is possible to speculate on the basis of these find-

ings that the capacity of the developing newborn to switch from solely IgM-producing capability to IgM plus IgG synthesis is to some extent linked to the appearance of an Ia⁺ B cell subpopulation. Finally, there is also some evidence that a C3 receptor surface marker may be important in determining the ontogenetic development of B cell activation [42]. The results of several studies have indicated that the C3 receptor is a component in the cellular pathway leading to T cell-dependent, but not T-independent antibody responses [137, 257, 362, 363]. Ontogenetic studies show that the C3 receptor-bearing subpopulation of IgM⁺ B cells does not appear in significant numbers until approximately 10 days of age. The appearance of a C3⁺ B cell subpopulation, therefore, would seem to correlate closely with the onset of capacity to produce antibodies to T cell-dependent antigens in the developing newborn.

There is little doubt that the development of immunocompetence in the newborn is to some considerable extent a reflection of qualitative changes in functionally distinct B cell subsets. However, as recently pointed out by Mosier et al. [307] the accumulated data on functional maturation of B cells suggest that by 2 weeks of age in the mouse, the state of B cell differentiation is probably not qualitatively limiting for antibody synthesis. The delayed appearance of adult-like immune reactivity to T cell-dependent and TI 2 antigens until 4–6 weeks of age would thus strongly suggest that additional factors are controlling the expression of immune responses during ontogeny.

IV. Ontogeny of Macrophages Involved in Immune Functions

Macrophages are known to play important roles in the induction and regulation of most types of humoral and cell-mediated immune functions [22, 287, 429, 481]. The selective depletion of macrophages from lymphocyte populations by plate adherence, carbonyl iron and magnet, antimacrophage serum plus complement, and by passage over Sephadex G-10 columns has been shown to drastically diminish or completely abrogate various immune functions [92, 247, 255, 303, 404]. Restoration of these immune reactivities could be accomplished by reconstitution with syngeneic macrophages. A more recently defined role for macrophages relates to the propensity of this class of cell to bind various T cell-derived regulatory factors [134, 136, 380, 469] and to transmit immunoregulatory signals to other cells [378]. Although both live and dead macrophages bind T cell factors, only live macrophages are able to successfully pass on the immunoregulatory

signals [154, 381]. On the basis of these findings the authors suggest that the role played by macrophages in relaying T cell communication signals is as important as their antigen-presenting functions. Macrophages are also known to produce their own immunosuppressive and stimulatory factors [64, 240]. Macrophage-lymphocyte interactions have been shown to be genetically restricted to the I region of the major histocompatibility complex [130, 405]. Moreover, macrophage populations which differ in their expression of Ia [106] may represent functionally distinct subsets involved in the induction of antibody synthesis [330] and in antigen-specific T cell proliferation [105].

Moore and Metcalf [291] have established that differentiated cells of the granulocytic series are absent in the murine yolk sac prior to 10 days gestation. In a study of the origin of cells of the mouse mononuclear phagocytic system, *Cline* [96] has shown that colony-forming cells (CFC) consisting of typical granulocytes and macrophages [97] can be generated in vitro in the 7- to 9-day-old yolk sac. Cell suspensions from embryos younger than 7-10 days did not form CFC. Fetal liver cells at 12-14 days of gestation were shown to give rise to functionally active macrophages following 6 days of culture in diffusion chambers [96]. Thus, mature macrophages as defined by phagocytic and immunoglobulin G receptor activity were observed in 10-day-old yolk sac cell suspensions and in 12-day-old fetal liver [96]. These findings suggest that the appearance of macrophages is a very early event in murine ontogeny. However, it has been suggested that the poor antibody responsiveness of newborn mice [138, 442, 443] is due in part to a lack of functional macrophages [21, 41, 48, 187]. This conclusion is based on findings that (a) neonatal mice are capable of increased antibody synthesis if given adult macrophages along with antigen [21, 41, 48], and (b) that peritoneal exudate cells (PEC) from newborn mice do not reconstitute antibody responsiveness to sublethally irradiated adult recipients while adult macrophages do [187]. However, with the possible exception of one particularly well-controlled study [48] attempts to attribute restorative effects of adult PEC's, known also to contain significant numbers of lymphocytes, solely to macrophage activity is questionable. Moreover, conclusions based on adoptive transfer of thioglycolate-induced neonatal PEC's [187] must be viewed in terms of earlier findings showing that adult PEC's induced with this agent failed to augment antibody responses in newborn mice [41], whereas unstimulated adult PEC were quite effective. *Landahl* [242] has shown that splenic adherent cells from newborn mice fail to synergize with adult splenic nonadherent cells to produce antibody until the 5th day after birth. The subsequent

increase in spleen adherent cell activity appeared to parallel the development of antibody responsiveness in the developing intact animal. These findings support the contention that a relative lack of macrophage function may be a contributing factor to the low levels of antibody synthesis in young animals. Other investigators, however, have concluded that the macrophages required for antibody synthesis are present in newborn mice [138, 387]. In reconstitution experiments in vitro employing reciprocal recombinations of adult and young macrophage-rich adherent cells and lymphocyte-rich non-adherent cells, it was found that the cellular deficit in young animals resided in the nonadherent lymphocyte-enriched fraction and not in the adherent population [138]. Also, *Rabinowitz* [387] demonstrated that addition of adult PEC to newborn spleen cell cultures failed to enhance either DNA synthesis or the primary plaque-forming cell response to SRBC. In addition, heat-killed macrophages, which themselves are incapable of antigen-processing, enhanced both the in vivo and in vitro antibody response of neonatal spleen cells [379] further indicating that the poor responsiveness of the newborn cannot be solely attributed to a defect in antigen processing and presentation. Using an antigen-specific macrophage-dependent T cell proliferation model *Lu et al.* [260] have recently presented evidence that the capacity of macrophages to interact with T lymphocytes effectively did not occur until 3-4 weeks after birth. The results suggest that the failure of macrophages from newborn mice to stimulate adult T cell proliferation is due to a relative deficit in numbers of a subpopulation of Ia-bearing macrophages [260].

Like most other cellular systems, macrophages have now also become subject to subset analysis. It is clear that only macrophages of a particular type with Ia-antigens expressed on their surface can function as proper antigen-presenting cells. Moreover, spleens of newborn mice are relatively deficient in the subpopulation of adherent Ia⁺ dendritic cells [446, 447] which are known to be particularly effective stimulants of allogeneic [448] and syngeneic [336] mixed lymphocyte reactivity. Whether this is entirely because of a delayed development of such cells in young animals or due to the presence of other suppressing cells remains to be settled.

V. Immunoregulation in the Fetus and Newborn

A. Evidence for Suppressor Cell Activity

Ever since *Gershon and Kondo* [155, 156] first proposed that many immune responses are under the regulatory influences of thymus-derived T

lymphocytes, a prodigious amount of information has accumulated describing suppressor cell activities both in vivo [152, 288] and in vitro [120, 238]. Suppressor cell effects have been largely attributed to T cell populations [152, 155, 156, 288]; however, there is also evidence that B cells [117, 118, 221, 503] and macrophages [128, 129, 141, 142, 225, 232, 372] can mediate inhibitory functions. A variety of agents have been shown to activate suppressor cells including mitogens [116, 394], microbial substances [43, 59, 213, 413], anti-antibodies [127, 209], hormones [400], drugs [54], and chronic UV irradiation [140], in addition to alloantigens [89, 395] and a multitude of conventional laboratory antigens, when used at appropriate concentrations [68, 120, 121, 237, 238]. Many suppressor cell systems, once activated show exquisite specificity towards the stimulating antigen [152] while other spontaneous [32, 227, 489] or induced [62, 212, 326, 354] suppressor cells exhibit nonspecific effects. Murine suppressor T cells (Ts) have been shown in most experimental systems, to express the common cell surface antigen phenotype $\text{Lyt } 1^{-}23^{+}$, Ia^{+} [278]. In some cases, however, inhibitory T cells have been reported to have an $\text{Lyt } 1^{+}23^{-}$ [354, 391, 490] or $\text{Lyt } 123^{+}$ [366] phenotype. It is not yet certain whether these findings indicate the existence of truly nonconventional suppressor cell populations, with respect to Lyt phenotype, or that certain experimental designs inadvertently focused on an inductive or amplifying cell involved in the generation of conventional $\text{Lyt } 1^{-}23^{+}$ suppressors [69, 133]. In any case it is important to realize that the term 'suppressor cell' has taken on a more restricted and precise definition in view of recently obtained knowledge regarding the complexity of cell-cell interactions required to obtain the actual final effector(s) of suppression [69, 75, 122, 123, 273]. For example, immune $\text{Lyt } 1$ cells can help thymus-dependent B cells produce antibodies, and they can also serve as efficient inducers of $\text{Lyt } 23$ suppressor cells by interacting with a nonimmune set of $\text{Lyt } 123^{+}$ acceptor cells [75, 123, 273]. This regulatory pathway is known as a feedback inhibition circuit since one of the eventual targets for $\text{Lyt } 123^{+}$ cell-associated suppression is an $\text{Lyt } 1^{+}$ inducer cell [69]. Moreover, regulatory cells within or between such feedback circuits may communicate by secreting specific factors [218, 239, 296, 467, 504] which can be transmitted by macrophages [378] to the next cellular target in the sequence. Antigen-specific Ts [324, 338, 376] and Ts-derived suppressor factors (TsF) [174, 465, 472] have been shown to frequently bear determinants encoded by the I-J subregion of the major histocompatibility complex (MHC). It has been proposed that these I-region determinants serve as restrictive communicative links allowing the correct order of cell-cell interaction to take place within

an immunoregulatory circuit [468]. The chain of events in a regulatory loop extending from the original stimulus of a suppressor circuit to the ultimate feedback suppression of immune reactivity may involve the sequential activation of two or more sets of phenotypically distinct Ts and TsF [150, 468]. Thus, many of the 'suppressor cells' and 'suppressor factors' that have been previously described are probably not the actual effectors of suppression, but instead represent intermediaries of a long, complex chain of events leading to the final effectors of suppression. Recent analysis of Ts pathways in delayed-type hypersensitivity to the *p*-azobenzenearsonate (ABA) hapten has, for instance, revealed that the antigen-induced 'first order' suppressor cell bears a particular idio type, while subsequent factor-induced 'second order' suppressor cells express anti-idio type receptors [464]. This and other recent findings [124, 197] are in general agreement with the hypothesis that T cell circuits may establish idiotypic-anti-idiotypic regulatory pathways [464]. Neonatal mice are known to have a well-developed feedback-inhibitory circuit [318, 379] and lymphocytes from neonatal mice can suppress antibody responses of adult lymphocytes to phosphorylcholine via auto-anti-idio type suppression [458]. It is therefore likely that fetal/neonatal immunoregulatory pathways may prove to be as precise and complex as those presently being defined in the adult.

1. Suppression of Antibody Synthesis

As previously noted newborn mice acquire the ability to produce antibodies to most T cell-independent antigens at a very early stage in postnatal development [112, 186, 277, 301, 305, 307, 387], while responsiveness to T cell-dependent antigens usually appears later [138, 186, 387, 442, 443, 470]. *Mosier and Johnson* [305] initially interpreted these findings as indicating that B cells mature earlier during ontogeny than do T cells in terms of their functional roles in antibody formation. Their experiments to test this reasoning in which an excess of adult lymph node cells were added to antigen-stimulated neonatal spleen cell cultures showed, however, that mature adult T cells could not restore the poor responsiveness of newborn spleen. Prior removal of T cells from neonatal spleen with anti-Thy-1 and complement was necessary to allow the remaining neonatal B cells and macrophages to respond in culture when excess adult T cells were added. In addition, small numbers of neonatal spleen cells were shown to significantly suppress antibody responses of adult spleen cells to the T cell-dependent antigen, SRBC as well as to the T-independent antigen DNP-Ficoll. The suppressive effect by newborn spleen cells was found to be associated with cells passing

through nylon wool columns, and was abrogated by anti-Thy-1 and complement treatment. From these findings it was concluded that the poor antibody responsiveness in newborn animals is at least partly due to an excess of endogenous suppressor T cells which can interfere with helper T cell functions and also negatively influence T-independent B cell functions [305]. This conclusion was strengthened by subsequent studies showing that neonatal thymocytes constitute a rich source of suppressor cells for adult antibody synthesis [308]. A considerable enrichment of mouse thymus-derived suppressor cells was achieved by infection of newborn animals with mouse thymic virus [102]. The residual T cells remaining after infection, representing about 5% of the normal thymus T cell number were highly enriched for suppressor activity and retained PHA and ConA reactivity, but had lost all ability to respond in MLC and CML assays [308]. The population of mouse thymic virus-resistant T lymphocytes containing the neonatal inhibitory cells were characterized as large cells with high DNA content and were localized to the subcapsular outer cortex of the thymus. In addition, these cells were found to be less susceptible to killing with anti-TL and anti-Thy-1 sera and more susceptible to killing with anti-H-2^b serum that was the average normal thymocyte. It was inferred in these studies that suppressor cells residing within the residual 5% virus-resistant outer cortical thymocytes emigrate to the neonatal spleen where similar immunosuppressive activity was detectable [308].

The thymic origin of the nonspecific suppressor cells which appear early in ontogeny was further substantiated by studies showing that unprimed fetal thymocytes efficiently suppressed the T-dependent primary antibody response of adult syngeneic spleen cells to SRBC [261]. However, contrary to the mouse thymic virus-resistant suppressors which appeared to be of cortical origin [308], the fetal thymus-derived suppressor cells demonstrated characteristics of a thymic medullary lymphocyte.

The fetal suppressor T cell was shown to have a low content of Thy-1 antigen and was not sensitive to treatment with hydrocortisone [261]. Studies by *McCullagh* [270, 271] performed in the rat also indicate that antibody responsiveness during the neonatal period is regulated by an active thymus-derived T cell suppressor mechanism. It was demonstrated that the impaired capacity of newborn rats to mount an anti-SRBC response could be restored by adoptive transfer of adult thoracic duct lymphocytes only if the neonatal recipients were first thymectomized [270] or exposed to low dose of irradiation [271]. Antibody-forming cell precursors were shown to be plentiful in the newborn rat. These findings were interpreted as indicating

that the failure of newborn rats to sustain adoptive responses was not due to inadequate neonatal macrophage function, but to an active newborn regulatory mechanism mediated by radiosensitive suppressor T cells of thymic origin [270, 271]. The suppressor T cells residing in murine neonatal spleen and thymus have also been characterized by *Mulder et al.* [310] as being sensitive to both X-irradiation and mitomycin C. *Calkins and Stutman* [65] have presented evidence suggesting that the suppressor cell population in newborn mice which inhibits antibody synthesis of adult lymphocytes in culture [301, 305] is distinct from the suppressor cells which can be found in adult lymphoid tissues [66, 122]. Newborn suppressors were thus shown to differ from adult inhibitory T cells in terms of an apparent absence of antigen specificity, lack of requirement for stimulation from primed cells to suppress, and a relative insensitivity to cytotoxic treatment with anti-Thy-1 and complement.

There is at present very little detailed information concerning the mechanism(s) of action of the fetal and neonatal cells which inhibit antibody synthesis. There is some evidence to suggest that neonatal suppressors act *in vitro* during the early inductive phase of the antibody response [261] and that suppression may be by cell contact rather than through the elaboration of soluble suppressive factors [310]. The question of whether the primary target for neonatal T suppressors is a B cell or another T cell has not been resolved. *Mosier et al.* [308] presented evidence that neonatal T cells can exert a negative regulatory influence directly on B cells responding to the T cell-independent antigen TNP-Ficoll by reducing the number of B cells initially triggered by antigen. Neonatal T cells have also been shown to suppress other T-independent antigens including, type III pneumococcal polysaccharide [294], the synthetic polypeptide poly-(*D*-Tyr, *D*-Glu)-poly(*d*-Pro)-poly(*D*-Lys) [188], and DNP-PAA (polyacrylamide beads) [112]. However, studies involving careful dilution analysis of suppressor T cell effects on T-dependent versus T-independent antigens indicate that T-dependent antibody synthesis is much more sensitive to suppression by fetal or neonatal cells than is T-independent antibody synthesis [261, 318]. Thus, *Luckenbach et al.* [261] have shown that fetal thymocytes suppress the *in vitro* antibody response of adult spleen against SRBC when present in thymus to spleen cell ratios of 1:5 to 1:500, whereas no suppression was observed on the antibody response to the TI antigen NIP-POL even at a fetal thymocyte to adult spleen cell ratio of 1:5.

We have examined the immunoregulatory effect of purified splenic T lymphocytes from newborn mice on *in vitro* antibody synthesis to the thymus-dependent antigens SRBC and DNP-KLH, and on the thymus-

independent antigens DNP-Ficoll and DNP-KLH [318]. In these experiments T lymphocytes were isolated from the spleens of 5- to 8-day-old or 6- to 8-week-old adult CBA/H mice by affinity fractionation on Ig-anti-Ig glass bead columns [493]. The purified T cells were then added in graded numbers to assay Marbrook cultures containing 20×10^6 normal adult CBA/H spleen cells and optimal immunizing doses of TD and TI antigens. Antibody synthesis was measured after 4 days of culture as antigen-specific IgM PFC. The results presented in table I show that the addition of 1×10^6 newborn spleen T cells, representing 5% of the total cell population in the assay culture, strongly inhibits antibody synthesis to the two TD antigens, SRBC and DNK-KLH. Significant inhibition of TI antibody synthesis was observed in only a few instances (2 out of 7 experiments) and then only when the ratio of newborn T to adult spleen cells was 1:20 or higher. Thus, dilution analyses showed that suppression of TI responses occurred only in the presence of relatively high numbers of newborn T cells, whereas TD responses were consistently inhibited by 10^4 (0.05% of the total cell population in assay cultures) and sometimes by as few as 10^3 newborn T cells. It is notable that the suppression of TI responses by newborn lymphocytes in vitro reported by others [112, 308] was shown only to occur at a ratio of newborn to adult cells much higher than 1:5. It is our experience that normal adult T cells begin to exert inhibiting effects in control cultures when added in excess of 20% of the total cell population in the present culture system. Nevertheless, we conclude that it is likely that newborn T cells, and/or an as yet undefined contaminating cell subpopulation, can exert inhibitory effects on TI antibody responses but with low efficiency. However, the prevailing inhibitory effect mediated by newborn T lymphocytes on antibody synthesis in vitro is directed towards TD responses. These findings could be interpreted as indicating that newborn inhibitory T cells either directly suppress TD B cells more efficiently than they do TI B cells [257], or that helper T cells required for TD antibody synthesis are primary targets for suppression. Our preliminary evidence tends to favor the latter possibility. Thus, newborn T cells have been shown to exert helper effects for TD antibody synthesis when added to adult T cell-depleted spleen B cell cultures, while causing strong suppression of antibody production in parallel cultures of whole adult spleen cells [Stegagno and Murgita, unpublished observations].

Negative selection experiments were performed with various anti-T cell reagents plus complement to determine the cell surface antigen phenotype of the newborn inhibitory T cell. Purified splenic T cells from 5-day-old

Table 1. Inhibitory effect of newborn splenic T lymphocytes on T cell-dependent versus T cell-independent antibody responses in vitro

Source of splenic T cells Added to assay culture	number of T cells transferred to assay culture	Assay culture			
		I _g M PFC/culture \pm SEM (% suppression)		DNP-specific PFC	
		SRBC-PFC		DNP-KLH	DNP-Ficoll DNP-POL
T _{adult}	—	1,140 \pm 260	1,181 \pm 74	768 \pm 79	1,176 \pm 63
T _{newborn}	10 ⁶	1,146 \pm 96 (0)	1,758 \pm 38(0)	952 \pm 79(0)	1,740 \pm 82 (0)
T _{newborn}	10 ⁶	452 \pm 35 (60)	591 \pm 45(50)	460 \pm 36(40)	1,140 \pm 95 (3)
T _{newborn}	10 ⁶	653 \pm 148(43)	678 \pm 34(43)	695 \pm 20(10)	1,127 \pm 54 (4)
T _{newborn}	10 ⁶	690 \pm 80 (39)	520 \pm 26(56)	817 \pm 44(0)	1,165 \pm 66 (0)
T _{newborn}	10 ⁶	1,180 \pm 160(0)	673 \pm 22(42)	830 \pm 40(0)	1,060 \pm 772(8)

Ig-anti-Ig column purified splenic T cells [493] from 5- to 8-day-old CBA/H mice were added in graded numbers to secondary Marbrook assay cultures containing 20×10^6 normal adult CBA/H spleen cells and optimal immunizing doses of the T cell-dependent antigens SRBC (3×10^6), and DNP-KLH (0.1 μ g/ml), and the T-dependent antigens DNP-Ficoll (10 ng/ml) and DNP-POL (1 μ g/ml). Antigen-specific IgM plaque-forming-cell (PFC) responses were measured after 4 days of culture. Data are expressed as the mean number of PFC \pm standard error of the mean of triplicate cultures. DNP-specific PFC were enumerated using TNP coupled SRBC as indicator cells [see reference 314 for details].

Table II. Cell surface Lyt differentiation antigen phenotype of newborn splenic inhibitory T cells

Source of splenic T cells	Cytotoxic treatment with antisera and complement	Assay culture, % of control PFC response (mean \pm SEM of 5 experiments)
-	-	99 \pm 5
T _{adult}	-	44 \pm 2
T _{newborn}	-	46 \pm 2
T _{newborn}	+ RC	98 \pm 5
T _{newborn}	anti-T + RC	75 \pm 2
T _{newborn}	anti-Thy-1.2 + RC	99 \pm 7
T _{newborn}	anti-Ly 1 + RC	42 \pm 3
T _{newborn}	anti-Ly 2 + RC	

Ig-anti-Ig column purified splenic T cells from newborn CBA/H mice were exposed to anti-T (rabbit anti-mouse brain antiserum), anti-Thy-1.2 (AKR anti-C3H), anti-Lyt 1.1 and anti-Lyt 2.1 antisera plus rabbit complement (RC). Following selective lysis with these antisera the remaining cells were adjusted such that the lymphocyte density in the untreated control was 1×10^5 viable cells per ml. One ml aliquotes of cells were transferred to adult CBA/H spleen cell assay cultures immunized with SRBC. The number of day-4 IgM anti-SRBC PFC in control cultures without added T cells was 665 ± 56 .

CBA/H mice were exposed to rabbit antimouse brain (anti-T), anti-Thy-1, anti-Lyt 1.1 and anti-Lyt 2.1 sera plus rabbit complement. Following selective lysis with these antisera the remaining cells were tested for their ability to induce suppression of TD anti-SRBC response in secondary assay cultures. As shown in table II, the ability to cause suppression was eliminated by cytotoxic pretreatment with anti-T, anti-Thy-1, anti-Lyt 1.1, but not by anti-Lyt 2.1 sera. Absorption studies verified that the elimination of suppressor activity was, in fact, due to the effect of specific anti-Lyt 1.1 antibodies, and additional phenotyping experiments were performed with other mouse strains with essentially identical results [318]. Thus, lymphocytes capable of inhibiting TD antibody responses residing in the spleen of newborn mice are T cells with an Lyt 1+2- phenotype [167, 318]. We further analyzed the Ia antigen phenotype of these newborn inhibitory T cells, as such markers have been found to be of importance in delineating various T cell subsets in other systems [133, 323, 339, 466]. Splenic T lymphocytes from 2- to 4-day-old CBA/J mice were exposed to anti-T cell sera, anti-H-2 κ , and to various anti-Ia sera specific for subregions within MHC I re-

gion. After selective lysis in the presence of rabbit complement the cells remaining were tested for their ability to suppress anti-SRBC antibody synthesis of adult normal spleen cell cultures. The results presented in table III show that the inhibitory activity of newborn T cells is effectively abrogated by anti-I^k, anti-I-ABJ^k, anti-I-J^k, but not by anti-I-EC^k, or by anti-I-J^b. The I-J^k determinant is therefore expressed on newborn CBA/J splenic inhibitory T cells [200, 319].

While the presence of an I-J marker is known to be associated with T cells possessing suppressor properties, the expression of the Lyt 1+2- phenotype on the newborn inhibitory T cells is not compatible with conventional suppressor effector cells which are usually Lyt 1-2+ in phenotype. It is likely, although as yet unproven, that the Lyt 1+2- newborn inhibitory T cells represent a population of 'suppressor inducer' cells, rather than being truly non-conventional effector suppressor cells. This premise is based on evidence by Ptak et al. [379], that newborn mice can be shown to contain considerable numbers of 'latent' helper cells when overriding suppressor mechanisms are deliberately interrupted. Moreover, this study revealed distinct similarities between neonatal helper cells and a subpopulation of adult helper T cells which have powerful suppressor-inducing abilities. These findings would therefore indicate that neonatal Lyt 1+2-, I-J+ regulatory T cells may be comparable to a certain type of adult Lyt 1 helper cell which is an efficient inducer of feedback inhibition [69]. An alternative possibility, albeit less likely, is that the newborn Lyt 1+ inhibitory T cell we have described is an actual specialized effector cell which operates outside previously described feedback circuits [69] to help control immune reactivity during ontogeny. It is interesting to note in this regard that while young NZB mice show an unusually early appearance of immunocompetence [132, 369], which may be linked to an absent Lyt 123+ cell-associated feedback suppression circuit [76], newborn NZB spleen cells still retain active inhibitory cell activity as measured in vitro [246].

There is now substantial evidence for the presence in newborn mice of an unusually active population of T cells with efficient ability to suppress T cell-dependent antibody synthesis in vitro. While the mechanism of suppression may be direct or indirect, it is likely that helper T cell function is a primary target for the inhibitory process. Whether these inhibitory cells differ in number or activity per cell from similar T cells in adult individuals is not known. Newborn T cells would also seem able to exert less effective suppressive activity on certain relatively-T-independent antibody responses. The actual reason(s) for the presence of active inhibitory cells in the neonate

Table III. Expression of I-J determinants on CBA/J newborn splenic inhibitory T cells

Source of T cells	Treatment antisera + RC	H-2 region detected						Assay culture I _g M SRBC PFC/ culture \pm SEM (% suppression)
		K	I-A	I-B	I-J	I-E	I-C	
T _{adult}	-							652 \pm 47
T _{newborn}	-							886 \pm 62(0)
T _{newborn}	-							384 \pm 55(41)
T _{newborn}	RC							360 \pm 50(45)
T _{newborn}	anti-T							748 \pm 64(0)
T _{newborn}	anti-Thy-1							576 \pm 76(12)
T _{newborn}	C3H. SW anti-C3H							732 \pm 12(0)
T _{newborn}	A. TH anti-A. TL	K	K	K	K	K	K	840 \pm 81(0)
T _{newborn}	(A. THXBIO. HTT) F ₁ anti-A. TL		K	K	K			780 \pm 40(0)
T _{newborn}	BIO. A (3R) anti-BIO. A (5R)				K			756 \pm 43(0)
T _{newborn}	BIO. S (7R) anti-BIO. HTT					K	K	372 \pm 21(43)
T _{newborn}	BIO. A (5R) anti-BIO. A (3R)				B			384 \pm 52(41)

Ig-anti-Ig column purified splenic T cells from 2- to 4-day-old CBA/J mice were treated with anti-Ia subregion specific alloantisera (prepared at the University of Toronto by Dr. T. L. Delovitch) plus rabbit complement (RC). The cells remaining after cytotoxic pretreatment were adjusted to a viable lymphocyte count of 10^6 per ml in the untreated control. Aliquots of pretreated T cells, containing the equivalent of 10^6 cells minus the number selectively killed, were transferred to assay cultures consisting of 20×10^6 syngeneic adult spleen cells plus 3×10^6 SRBC. Day-4 IgM anti-SRBC PFC are expressed as the mean number of PFC \pm standard error of the mean of triplicate cultures.

is still a matter of speculation. In addition, we feel that the presence of other non-T suppressor cells has not been fully excluded by the available published evidence.

2. Suppression of Cell-Mediated Immunity

Olding and Oldstone [341] and *Olding et al.* [343] first observed that lymphocytes from cord blood of human newborns could effectively inhibit division of their mother's lymphocytes in vitro. In these studies equal numbers of viable lymphocytes from a mother and her male baby were mixed and cultured for 3 days with and without PHA. The mitogen was usually needed to get a sufficiently high number of dividing cells for chromosome analysis. Examination of mother-baby mixed cultures for the occurrence of male and female metaphases using the fluorescent Y chromosome technique revealed that 98% of the total cultured lymphocytes in mitosis were the newborn's, whereas less than 2% were from the mother [341, 343]. The same strong mitotic inhibition also occurred when lymphocytes from newborn male babies were mixed with lymphocytes from nonrelated recently delivered mothers and from nonrelated, nonpregnant women. *Lawler et al.* [243] reported a similar inhibitory effect by male newborn lymphocytes on parental cells in two-way mixed cultures. Subsequent cell fractionation studies showed that the suppressor activity in cord blood was associated with the T lymphocyte population [342].

We have further examined the immunologic consequences of the interaction between lymphocytes from human newborns and their mothers in vitro [344]. The cellular proliferation of mother-baby in two-way mixed lymphocyte reactions was assessed by simultaneously measuring ^3H -thymidine incorporation and the number of cells in mitosis containing the Y chromosome. The results demonstrated that the mitotic inhibition of maternal cells can be readily detected in 6-day two-way mixed cultures of equal numbers of newborn and maternal lymphocytes. Approximately 94-96% of the total cultured cells contained the Y chromosome, indicating that most of the dividing cells in the cocultures were of newborn origin. As shown in table IV, the strong suppressive effect mediated by actively dividing newborn lymphocytes was converted to a stimulatory effect on maternal lymphocyte proliferation by irradiation of the newborn cells. We also sought to determine whether the mode of action of newborn inhibitory lymphocyte was via cell-cell contact or through the liberation of a soluble inhibitor factor(s). Experiments were performed using double chamber Marbroo cultures [135] in which newborn suppressors and adult target lymphocyte

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Table IV. Lymphocytes from human newborns must be viable to suppress proliferation of their mother's lymphocytes in the mixed lymphocyte reaction

Case No.	Culture time, days	Uptake of ³ H-thymidine by lymphocytes from			% of metaphase cells having a Y chromosome	Stimulation index
		mother	newborn male	mother + newborn		
1	6	2,734 ± 209	7,935 ± 501	18,988 ± 1,534	96	1.8
2	6	1,050 ± 178	2,323 ± 613	10,662 ± 985	94	3.2
3	6	3,554 ± 328	*447 ± 12	21,699 ± 1,724	0	5.4
4	6	1,893 ± 144	*380 ± 129	14,919 ± 1,456	0	6.6

* Lymphocytes from newborn male babies were irradiated with 6,000R before co-culture with maternal cells.

Lymphocytes, obtained from cord blood of newborn male babies and from peripheral blood of their mothers, were enriched by Ficoll-Isopaque gradient centrifugation, and suspended in RPMI medium supplemented with 10% pooled AB serum. 2×10^5 lymphocytes from the mother and baby were cultured alone, and co-cultures contained 1×10^5 cells from each individual. For Y chromosome analysis cells from multiple cultures were pooled, treated with colchicine for 2 h, disrupted, fixed, and stained with quinacrine-dihydrochloride [for details see Olding et al. 343]. Maternal lymphocytes survived as well as newborn lymphocytes in this culture system, and maternal cells cultured alone were able to enter mitosis normally. Cases 1 and 2 demonstrate a mitotic inhibition of maternal cells as the result of co-culture with an equivalent number of viable baby lymphocytes. In cases 3 and 4 the inhibitory effect of newborn lymphocytes on maternal cell proliferation is abrogated when baby cells are irradiated with 6000R prior to co-culture.

could be separated by Nucleopore or dialysis membranes. PHA was added to both lymphocyte compartments. After 3 days of culture, the newborn cells in the inner chamber, and maternal cells in the outer chamber of Marbrook vessels were pulsed with ³H-thymidine. The amount of radioactivity incorporated by maternal lymphocytes exposed to the dialyzable products of newborn lymphocytes was compared to control cultures consisting of maternal cells cultured alone. Our findings summarized in table V show that mitogen-stimulated cells from newborns could suppress the proliferation of lymphocytes from recently delivered mothers even when separated by a dialysis membrane. PHA-stimulated lymphocytes from nonrelated adults did not inhibit division of maternal lymphocytes, or lymphocytes from another nonpregnant woman when physically separated in double chamber cultures. Moreover, lymphocytes from one newborn failed to inhibit the proliferation of lymphocytes from another newborn. Our findings thus indicated that proliferating newborn lymphocytes induce mitotic inhibition

Table V. Human newborn lymphocytes secrete a low molecular weight factor inhibitory for maternal lymphocyte proliferation

Case No.	Lymphocytes from	Exposed to dialyzable product(s) from	³ H-thymidine incorporation (cmp ± SEM)	% suppression
1	mother	—	148,697 ± 19,123	
		newborn	42,135 ± 514	71
2	mother	—	106,454 ± 1,658	
		newborn	54,987 ± 873	48
3	mother	—	107,225 ± 4,298	
		newborn	43,829 ± 1,254	59
4	mother	—	81,945 ± 2,156	
		nonpregnant female	75,515 ± 988	8
5	nonpregnant female	—	191,180 ± 1,957	
		nonpregnant female	231,615 ± 1,898	0
6	newborn	—	265,179 ± 9,426	
		newborn	267,792 ± 4,970	0

Using a Marbrook double chamber culture system [344] 4×10^6 newborn lymphocytes were separated from 8×10^6 maternal lymphocytes by a dialysis membrane. PHA was added to both compartments in a concentration of $50 \mu\text{g/ml}$. After 3 days of culture the cells in both compartments were harvested and 1×10^5 viable lymphocytes were transferred to microplate wells and pulsed for 5 h with ³H-TdR. In all experiments the survival rates of the cells in both chambers were equivalent.

of adult cells by releasing a low molecular weight suppressive factor(s). Other studies have also subsequently shown that cord blood lymphoid cells secrete soluble factors capable of inhibiting mitogen and alloantigen-induced proliferation of adult cells [495, 496]. Oldstone et al. [345] have further characterized the inhibitory cells in cord blood as a T lymphocyte subset bearing Fc receptors for IgG(T_G). This is consistent with findings that adult T lymphocytes with receptors for IgM(T_M) act as helper cells, while those with IgG receptors are efficient suppressors [293], and that the mean proportion of T_G cells in cord blood is about 3 times the adult mean [345]. More recent studies [115] indicate that the suppressor pathways in the human newborn may be more complex than earlier believed. Thus, newborn T_G⁺ cells suppress B cell maturation, but not B cell proliferation, while newborn T_G⁻ cells are strongly suppressive for T and B lymphocyte proliferation. Moreover, PWM-induced newborn suppressor T lymphocytes only inhibit the maturation of adult B cells when other T cells were present,

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suggesting that the newborn 'suppressor' is capable of inducing a secondary suppressor activity in the adult T cell population. It is possible that one of the inhibitory T cell subsets present in cord blood [115] may be analogous to the Lyt 1+ suppressor inducer cell in the murine feedback inhibition circuit [69].

The embryonic liver is known to be an early site of immunocompetence during murine ontogeny. *Globerson et al.* [163] have examined the question of whether the potential to suppress immune reactivity arises concomitantly with the appearance of immunocompetence in the liver. In initial studies mouse embryonic and neonatal liver cells were found to strongly suppress adult mixed lymphocyte reactivity and generation of cell-mediated lysis. The suppression occurred regardless of whether stimulator cells were syngeneic or allogeneic to the liver donor [163]. Newborn liver cells also suppressed GVH-induced mortality in sublethally irradiated F₁ mice challenged with parental spleen cells mixed with parental liver cells [162, 480]. Interference with GVH responses by neonatal liver cells could be demonstrated only in combinations where the injected spleen cells were syngeneic to the inhibitory liver cells. This genetic restriction for suppression of GVH in vivo contrasted with the findings earlier where MLR was inhibited by liver cells syngeneic or allogeneic with the effector cells [163]. Mouse embryonic liver suppressor cells have been characterized as belonging to a subpopulation of cells bearing receptors for the lectin peanut agglutinin [386].

Fetal mouse splenic, but not thymic cells have also been shown to reduce the ability of parental adult spleen cells to cause local graft-versus-host reactions in F₁ mice [432]. Suppression was still observed with spleen cells from newborn mice less than 24 h old, but disappeared completely by the time mice were 5 days of age. There was no requirement for histocompatibility between reacting and suppressor cells. It was inferred that the splenic suppressors were T lymphocytes since the inhibitory activity was completely eliminated by pretreatment with anti-Thy-1 serum [432]. In further studies by *Ptak and Skowron-Cendrzak* [377] it was shown that newborn mice sensitized to picryl chloride (PCL) within 24 h of birth fail to develop contact sensitivity when tested 4 weeks later. The reaction of animals sensitized at 2 days of age or later was always greater than in non-sensitized controls. It was postulated that painting with PCL at birth generates specific suppressor cells which after 4 weeks can still specifically interfere with the ability of the animals to respond to the original skin sensitizer [377]. Spleen lymphocytes of fetal or 1-day-old newborn mice were shown to suppress the passive transfer of contact sensitivity when injected along

with sensitized lymphocytes. Thymocytes were unable to affect the transfer reaction regardless of age of the cell donor, or the cell number used. Fetal mice were shown not to develop GVH reactions when given injections of parental lymphocytes. Moreover, fetal spleen cells were able to suppress the local GVH reaction elicited by immunized parental cells in F_1 recipients, and to reduce the severity and mortality rate of GVH in cyclophosphamide-treated F_1 recipients. These findings taken together imply that while antigen given in the perinatal period does not cause a specific immunization or sensitization, it may result in the triggering of an efficient antigen-specific suppressor mechanism.

Bassett et al. [34] have studied the inhibitory properties of newborn mouse splenic lymphocytes on a semiallogeneic mixed lymphocyte reaction. It was observed that stimulation of adult parental responder lymphocytes by newborn F_1 splenic lymphocytes was much lower than by genetically identical adult F_1 cells. The lack of stimulation was found not to be due to inadequate expression of MLR-activating antigens on the surface of newborn cells. Thus, mixtures of newborn and adult F_1 stimulator cells resulted in a reduction of proliferation of adult parental cells comparable to that observed when using newborn stimulators alone. It was concluded that newborn mouse spleen contains a suppressor which can inhibit adult responder cells in the MLR. Additional experiments showed that newborn F_1 cells cultures alone produce a factor capable of suppressing an adult parental anti- F_1 MLR [34]. These results were subsequently confirmed and extended by *Pavia and Stites* [356]. Their study also showed that irradiated newborn F_1 spleen cells failed to inhibit the proliferative response of maternal strain mice to PHA and Con A. Responses to LPS were suppressed by 55–60% compared to control cultures containing LPS-stimulated maternal cells alone. The newborn spleen suppressor activity for the semiallogeneic MLR was shown to persist for the first 2 $\frac{1}{2}$ weeks after birth and thereafter decayed quickly. The inhibitory activity of newborn spleen cells for the MLR was sensitive to cytotoxic pretreatment with heterologous antibrain associated T antigen [169] plus complement indicating that the suppressor is a T cell. Newborn spleen cells which did effectively suppress MLR were not able to interfere with the in vitro generation of cytotoxic lymphocytes in the CML assay. It was therefore suggested that newborn suppressor cells can selectively inhibit certain lymphocyte subpopulations involved in proliferative, but not cytotoxic reactions.

Argyris [23] has shown that T helper cells present in adult but not newborn spleen are effective in allowing in vitro proliferation of low numbers of

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adult mouse thymus cells to allogeneic spleen cells in vitro. Helper activity was totally absent in 6- to 8-day-old newborn spleen, partially present in 20-day-old spleen cells, and reached adult levels in the spleen of 40-day-old mice. Mixing neonatal spleen cells with adult spleen helper cells eliminated the adult helper activity for thymus-responding cells in the MLR. Thus, the lack of helper activity in newborn spleen cells is due to active suppression. Consistent with previously described neonatal suppressors of GVH [432] and contact sensitivity reactions [377], the inhibitory cell for MLR was found in the newborn spleen, but not in the thymus [23]. The inhibitory activity on neonatal spleen was unaffected by removal of glass-adherent cells, but was eliminated by removal of Thy-1⁺ cells, indicating that the suppressor cell is a T cell.

Further studies showed that in contrast to the findings of *Pavia and Stites* [356], the neonatal spleen suppressor T cell effectively inhibited both cell proliferation and the generation of cytotoxic cells in mixed lymphocyte cultures [24]. The suppressor activity is inversely correlated to the MLC reactivity in the spleen of young animals. Thus, spleens from mice up to 8 days of age have low MLR and strong suppressor activity. From 11 to 16 days of age spleens still have relatively low MLR, but they also have low suppressor effects. By 19 days of age there is full adult-like MLR and no suppressor activity is detectable.

Rollwagen and Stutman [402] have also observed a parallelism between the maturation of culture-generated suppressor functions in ontogeny and the immune functions they regulate in vitro. It is established that T cells with proliferative capacity to alloantigens are present at birth, whereas alloreactive cytotoxic cells appear later [367, 498]. Adult lymphocytes cultured in the absence of antigen for several days develop the ability to suppress the proliferative and cytotoxic response of noncultured syngeneic lymphoid cells to alloantigens [326]. Newborn liver and spleen cells precultured for 3-5 days were shown to be capable of suppressing MLR of fresh adult cells to a similar degree as precultured adult cells [401, 402]. However, unlike precultured adult cells, newborn precultured cells were not able to suppress the generation of alloreactive cytotoxic cells. Culture-generated suppressor cells for adult cytotoxic T cells were obtained from spleen and liver of animals only at 1 week of age or older. The serum requirements for the generation of culture-induced suppressors for MLR and CTL differed. Cells inhibitory for the MLR could be generated in horse serum but required fetal calf serum for their expression. Suppression of CTL generation was not dependent on any particular serum supplement [401].

Newborn inhibitory T cells of both the antigen-specific and naturally occurring 'nonspecific' types which suppress cell-mediated reactions have been classified in many systems as T lymphocytes [23, 24, 199, 356, 432]. Also, it has recently become apparent that newborn spleens may contain, in addition to T cells, other suppressor cells of a non-T type [199, 392, 402]. Recent studies by *Hooper and Murgita* [199] have defined a newborn splenic B lymphocyte-like inhibitory cell for primary MLR on the basis of (a) adherence to Ig-anti-Ig columns; (b) nonadherence to plastic dishes or Sephadex G-10; (c) insensitivity to cytotoxic treatment with anti-T cell reagents, and (d) differential agglutination with lectins known to selectively interact with B or T lymphocytes. Other studies have reported on the existence of non-T newborn suppressors with macrophage-like properties [402, or with characteristics distinct from classical B, T, macrophages or natural killer cells [398].

As in the case of the antibody-producing system, there is now convincing evidence for the existence of efficient inhibitory T cells in the neonatal mouse for various cell-mediated reactions. Whether these inhibitory T cells act in an independent manner or whether they interact with other cells in the formation of regulatory circuits is not yet clear. We would favor the latter view. We would also like to stress that studies of the murine newborn spleen populations have revealed a particularly dynamic system with regard to cellular composition and functional changes with time after birth. A further understanding of this dynamic state will require particularly stringent experimental designs and use of defined conditions to allow fruitful comparisons between results obtained by different laboratories.

B. Humoral Mediators of Suppression

The number of humoral immunoregulatory factors described in recent years has been enormous. Although the explosion of information in this area of immunology practically defies description, readers can refer to recent articles by *Nelson and Gatti* [327], *Waksman and Namba* [488], *Cooperband and Badger* [104] and *Kamo and Friedman* [217] which classify many of the known soluble immunoregulatory factors. With these comprehensive reviews as a background we will select and consider only certain literature which may be deemed pertinent to immune regulation in the fetus and newborn. Some emphasis will be placed on our own particular contributions to this area, namely the immunoregulatory role of alpha-fetoprotein [311].

There have been a number of studies describing immunosuppressive factors in normal mouse serum [58, 267, 328, 484]. While the inhibitory

factors in normal serum have not been fully characterized, there is a growing list of substances found in serum which have immunosuppressive properties in vitro. These include alpha-globulins [104, 382], C-reactive protein [297 to 299], lipoproteins [87, 295], alpha₁-acid glycoprotein [90], alpha₂-H globulin [57], interferons [439], and prostaglandins [171, 172, 385]. Unfortunately, the present lack of information concerning the ontogenetic development of many of these serum constituents precludes any speculation, even on a teleological basis, as to their possible immunoregulatory roles in the fetus and newborn.

Fetal calf serum, a commonly used serum supplement for tissue culture media, is known to be capable of significantly altering lymphocyte reactivity in vitro [143, 206, 223, 224, 228, 282, 348, 354, 358, 401, 426, 461, 475, 505]. Thus, nonspecific suppressor cell activity is induced by culturing mouse lymphocytes in the presence of fetal calf serum for 3–5 days [62, 212, 223, 326, 354, 401]. Fetal calf serum-dependent culture generated suppressors have been shown to affect antibody synthesis to thymus-dependent [62, 354] and thymus-independent [62] antigens, mixed lymphocyte reactivity [326, 401], and cell-mediated cytotoxic responses [223, 326]. The inhibitory cells activated by fetal calf serum appear to be T lymphocytes in many of the systems studied [62, 212, 354]. Fetal calf serum has also been shown to induce strong cytotoxic responses in both human [505] and murine [426] lymphocyte cultures. In addition to the immunosuppressive effects observed in vitro by fetal sera of human [26], bovine [223], and murine [313] origin, amniotic fluid, another regular feature of the mammalian fetal environment, has also been shown to have inhibitory properties both in vitro [131, 207, 258, 314, 478] and in vivo [337, 434, 474]. Mouse amniotic fluid (MAF) suppresses both T cell-dependent and T cell-independent antibody responses, in addition to lipopolysaccharide-induced polyclonal antibody synthesis [131, 314]. Amniotic fluids have been shown to effectively block mitogen reactivity, but not alloantigen-induced lymphocyte proliferation [258]. Sera from newborn mice [214, 313] and rats [491] are also inhibitory in vitro for T cell-dependent antibody synthesis [241, 313], mixed lymphocyte reactivity [241, 491] and mitogen responses [241, 491]. It is certain that fetal and newborn sera, and amniotic fluids contain more than one, and probably several substances capable of altering lymphocyte function in vitro in both positive and negative manners. For example, MAF has been shown to exert both suppressing and augmenting effects on mitogen- and alloantigen-induced lymphocyte proliferation in vitro [316]. Serum transferrin, present in MAF at a concentration of 300 μ g/ml, was found in its purified form, to cause significant lymphocyte stimulation [316]. In addition, part of the stimulatory activity of MAF

Table VI. Effects of low and high molecular weight components of mouse amniotic fluid on T cell-dependent and T cell-independent antibody responses in vitro

Preparation	IgM PFC/culture \pm SEM (% suppression)			
	SRBC-PFC	DNP-specific PFC		
		DNP-KLH	DNP-Ficoll	DNP-POL
NMS	1,150 \pm 132	1,191 \pm 121	665 \pm 44	770 \pm 62
MAF	330 \pm 62 (71)	337 \pm 95 (72)	298 \pm 32 (56)	374 \pm 24(52)
Dialyzed MAF	295 \pm 42 (75)	320 \pm 64 (73)	680 \pm 36 (0)	755 \pm 40(3)
PM10 retained MAF	367 \pm 81 (69)	475 \pm 110(61)	725 \pm 108(0)	689 \pm 60(11)
PM10 passed MAF	1,316 \pm 169(0)	1,030 \pm 185(13)	283 \pm 15 (58)	204 \pm 48(74)

Normal mouse serum (NMS), mouse amniotic fluid (MAF), dialyzed MAF, and the fractions of MAF which are retained ($> 10,000$ mol. wt.) and passed ($< 10,000$ mol. wt.) using an Amicon Ultrafiltration cell (Amicon Corp., Lexington, Mass.) fitted with PM-10 Diaflo membranes were added at $200 \mu\text{g/ml}$ to Marbrook cultures containing 20×10^6 CBA/H spleen cells immunized with TD (SRBC, DNP-KLH) and TI (DNP-Ficoll, DNP-POL) antigens. Specific antibody responses were measured as described in table I.

for MLR was shown to be in the low molecular weight dialyzable fraction. It has also been observed by us [314] and by others [131] that MAF exerts suppressive effects on both TD and TI antibody responses in vitro. However, as shown in table VI, the component in MAF which suppresses TI antibody synthesis is dialyzable, while a higher molecular weight moiety is clearly responsible for the MAF-mediated inhibitory effect on TD responses. Moreover, the fraction of MAF which is excluded by ultrafiltration on PM10 Diaflo membranes (which have 10,000 MW cut-off) suppresses TI, but not TD antibody responses. These findings show that MAF contains both low and high molecular weight components which are capable of altering lymphocyte functions in vitro. It is likely that a similarly complex array of immunoregulatory factors also exists in fetal, newborn, and pregnancy sera.

Fetuin, a major constituent of fetal calf serum, has been reported to have both stimulatory [176, 204, 383] and suppressive [500] effects on lymphocytes in vitro. Fetuin has been shown to stimulate the proliferation of hematopoietic stem cells in mice [236], to promote growth of bovine ovary cells in vitro [383], enhance rosette-forming human T lymphocytes [176], and to induce blast transformation and DNA synthesis of human peripheral blood lymphocytes in culture [204]. It would thus appear that in addition to serum

albumin [20, 371, 444] and transferrin [476, 477], fetuin is another serum component possessing significant growth-promoting properties for mammalian cells in vitro. Fetuin has also been reported to have immunosuppressive properties [282, 500]. For example, fetuin inhibits the ability of the isomitogenic mixture called H-PHA to induced lymphocyte transformation in a manner which can be reversed by increasing the dose of mitogen [282]. Since H-PHA can interact with fetuin and form complexes [500] it is assumed that fetuin-mediated inhibition of H-PHA responses can be attributed to competitive inhibition of the mitogen. However, it has also been shown that fetuin suppresses lymphocyte responses to L-PHA, an isomitogen which does not interact with fetuin [500]. This finding, together with other results describing the fetuin-mediated suppression of one-way-mixed lymphocyte reactivity [500], imply that fetuin may be able to exert a direct inhibitory effect on activated lymphocytes in vitro. It is notable, however, that only relatively high concentrations ranging from 1.25 to 2.50 mg/ml of commercially prepared fetuins were shown to be suppressive [500], whereas more purified fetuin was not significantly inhibitory at or below these concentrations [475]. Furthermore, commercial preparations of fetuin can be shown to be contaminated with AFP, a distinct embryonic substance [233] with proven immunosuppressive ability in vitro [311]. This would tend to leave some doubt as to whether fetuin itself possesses any significant immunosuppressive properties in vitro.

Mammalian alpha-fetoprotein, a glycoprotein of primarily hepatic origin [1, 148, 161] is a major constituent of serum and amniotic fluids during embryonic life [160, 268] and in murine species is still present in significant quantities in the newborn [347]. The levels of AFP then gradually diminish after birth to the low nanogram amounts normally present in adult serum [408, 409]. Aside from transient elevations in fetal-derived AFP which occur as a normal physiological event in the pregnant female, resynthesis of AFP in the adult usually reflects an abnormal expression of this embryo-specific protein signifying disease. Increased serum AFP levels have been found in a number of malignant and nonmalignant diseases [7] with high and sustained serum AFP concentrations being essentially diagnostic for primary liver cancer [1]. Molecules of AFP are composed of single polypeptide chains containing about 4% carbohydrate with a molecular weight of approximately 70,000 daltons [410]. Immunochemical analyses of AFPs from several different species have revealed both common and species-specific properties in their molecular structures. The similarities among the AFPs from various mammalian species are indicated by significant antigenic cross-reactions [210]. More-

over, the immunological cross-reactivity between the denatured peptides of AFP and albumin [406] support conclusions derived from sequence data [407] that these two serum proteins are structurally related and may even share a common ancestral gene. It is important to note that a number of different molecular subspecies of AFP have been detected by conventional polyacrylamide-gel electrophoresis [13, 177], extended agarose-gel electrophoresis [249], and by lectin [36, 435] and estradiol [482] affinity chromatography. Results in the murine system show that the increase in sialyltransferase activity in fetal liver with time of embryonic development correlates with the increased proportion of sialylated molecular variants of AFP [178, 506]. However, molecular variants of AFP have been shown to be partly, but by no means solely, due to variation in degree of sialylation [249, 250]. The biochemical basis of the extensive microheterogeneity remaining after desialylation is unknown.

AFP has thus been a well-studied 'onco-fetal' substance with regard to its physical-chemical properties, its normal and pathological distribution, and its diagnostic significance. However, until recently, very little had been learned about the possible biological functions of AFP. There is some evidence that AFP may function as an estrogen-binding receptor protein involved in protection of fetal tissues against circulating maternal estrogens [483]. However, strong estrogen-binding capacity appears restricted to AFPs of certain animal species [335] perhaps due to the presence of varying proportions of molecular subspecies of AFP with different estrophilic properties. It is therefore likely that AFP has other important functions in addition to its hormone-binding capacity. We have, for some time, been exploring the premise that AFP may play an important immunoregulatory role during ontogeny. The normal distribution of AFP during ontogenetic development would be consistent with such a function. Thus, in the murine system the physiological postnatal decline of serum AFP levels [347] is seen to correlate with the onset of adult-like immune reactivity.

A clear demonstration that highly purified AFP could suppress certain immune functions was initially obtained in the murine system [315, 316]. Mouse fetal-derived AFP was shown to exert a potent noncytotoxic inhibitory effect on primary IgM and secondary IgM, IgG and IgA anti-SRBC PFC responses *in vitro* [315]. Significant inhibition of TD primary antibody synthesis occurred with concentrations of AFP as low as 1.0 $\mu\text{g/ml}$. Dose-response studies revealed a differential inhibitory effect by AFP on the various immunoglobulin classes of antibody with IgA being most sensitive to suppression, IgG being moderately affected, and IgM responses the least

susceptible [315]. Lymphocyte proliferation induced by mitogens and allo-antigens was also shown to be prone to AFP-mediated suppression [316]. Thus, AFP in concentrations comparable to those shown to suppress antibody synthesis [315] effectively inhibits normal splenic lymphocyte proliferation by the T-cell mitogens Con A and PHA, the presumed B cell mitogen LPS, and mitomycin-treated allogeneic spleen cells in a one-way mixed lymphocyte reaction [316]. Studies by Zimmerman et al. [507] have confirmed that murine AFP isolated from amniotic fluid or fetal plasma is suppressive for in vitro anti-SRBC antibody synthesis. They further showed that the presence of sialic acid residues in the AFP molecule was necessary for immunosuppression. Sheppard et al. [423] demonstrated that AFP isolated from sera of hepatoma-bearing mice as well as amniotic fluid diminished primary in vitro antibody synthesis and reduced by 2-fold secondary antibody responses. Rat AFP of fetal [421] but not tumor [355, 421], origin was shown to suppress certain T cell reactions in vitro.

It would appear from our studies that the suppressive ability of AFP is preferentially expressed on the T cell-dependent limb of the immune response. Thus, IgA antibody synthesis which is considered highly dependent on thymus-derived T cells [98, 262] is the most sensitive to AFP-induced suppression, while IgM responses are least affected by AFP, and are also thought to be relatively T-cell independent. T cells which regulate B cell proliferative responses to LPS [333] are the likely targets for AFP-mediated suppression of LPS responses [316]. As pointed out earlier in this article, newborn mice can synthesize antibodies to many TI antigens within a few days after birth, whereas full antibody responsiveness to TD antigens is delayed for 4-6 weeks. If endogenous AFP does function in vivo to regulate antibody synthesis in the newborn mouse, then one would predict that TD antibody responses would be more susceptible than TI responses to AFP-mediated suppression. We therefore performed experiments to compare the inhibitory effects of AFP on in vitro antibody responses to the TD antigens, SRBC and DNP-KLH versus the TI antigens, DNP-POL and DNP-Ficoll [311, 314]. Shown in table VII are the results of a representative experiment demonstrating that AFP does effectively suppress specific antibody responses to TD antigens, but fails to significantly affect TI antigens. Polyclonal B cell antibody synthesis stimulated by LPS was also found to be unaffected by AFP [314]. These findings tend to exclude a direct inhibitory effect by AFP on B cell functions or on macrophages required for TI antibody synthesis [92, 247], and provide further evidence that T cells are a primary target for AFP-mediated immunosuppression. The additional find-

Table VII. Effect of AFP on T cell-dependent versus T cell-independent antibody synthesis in vitro

Preparation	Immunizing antigen	IgM PFC/culture \pm SEM (% suppression)		
		SRBC-PFC	DNP-specific PFC	
			SRBC-TNP ₄	SRBC-TNP ₄₀
NMS	SRBC	1,33 \pm 88		
AFP	SRBC	170 \pm 69(85)		
NMS	DNP-KLH		404 \pm 76	1,004 \pm 96
AFP	DNP-KLH		97 \pm 14(76)	321 \pm 58(68)
NMS	DNP-Ficoll		413 \pm 29	893 \pm 55
AFP	DNP-Ficoll		432 \pm 38(0)	799 \pm 24(11)
NMS	DNP-POL		168 \pm 18	417 \pm 34
AFP	DNP-POL		211 \pm 69(0)	434 \pm 42(0)

Normal mouse serum (NMS), and fetal-derived mouse alpha-fetoprotein (AFP) purified according to procedures described in detail elsewhere [315] were added at 200 μ g/ml to Marbrook cultures containing 15×10^6 CBA/J spleen cells and optimal immunizing doses of TD and TI antigens (see table I). DNP-specific PFC were detected using TNP coupled SRBC as indicator cells. TNP groups were coupled to SRBC in low (SRBC-TNP₄) and high (SRBC-TNP₄₀) epitope densities by mixing 4 mg and 40 mg of 2,4,6-trinitrobenzenesulfonic acid (Sigma Chemical Co.) per ml of packed SRBC [for details see reference 314].

ing in this study [314] that PHA responses were more effectively suppressed by AFP than were Con A responses, offered presumptive evidence for preferential action on certain T cell subpopulations as defined by these two T cell mitogens [455].

The selective nature of the in vitro immunoregulatory effects of AFP was further revealed in a series of experiments testing the impact of AFP on T cell proliferation towards defined histocompatibility-associated alloantigens [320, 359, 494]. In these studies the effect of AFP was tested on the four major genetic systems known to induce strong MLR activation in the mouse [360]: the Ia antigens, the serologically defined (SD) products of the major histocompatibility complex (MHC) K or D regions, the Mls locus products, and the products of an as yet undefined non-MHC system associated with a limited number of strains (i.e. DBA/2, B10.D2). The addition of AFP to mixed lymphocyte cultures at initiation could be shown to almost completely eliminate cell proliferation against Ia and/or M locus structures, while

normal numbers of T blasts were observed when stimulations were carried out against SD or non-MHC loci determinants [359]. It was possible to conclude from these findings that AFP exerts its suppressive activity on MLR through a selective interference with I region triggering systems. Moreover, AFP will not only usually fail to inhibit I region independent lymphocyte-activating systems, but actually will sometimes substantially augment these reactions. Subsequent analysis of the effect of AFP on cell-mediated lympholysis (CML) showed that the genetic relationship between responding and stimulating cells also largely predetermines whether or not AFP will inhibit the generation of CTLs [312, 361]. Thus, effector cells generated during lymphocyte activation with certain stimulator strains including DBA/2 and B10.G followed by testing for cytolysis on P815 tumor [312] or LPS-stimulated spleen cell targets [361] were found to be insensitive to AFP-mediated suppression. It is notable here that lymphocyte proliferation induced by non-MHC alloantigen systems on DBA/2 cells is not suppressed by AFP [359]. However, AFP did exert strong suppressive effects on CTL generation when using strain combinations with genetic differences in individual SD regions of the MHC [361]. The inhibition occurred independently of the effect of AFP on the MLC-activating phase, as normal numbers of T cells responded via cell division when confronted with SD antigens in the presence of AFP, but there was no subsequent generation of cytolytic T cells [361]. Since there is evidence for a necessary T-T cell collaboration between Lyt 1 helper cells responding against LD determinants and Lyt 2 CTL precursors reacting to SD determinants for effective generation of CTLs, it seems likely that AFP is blocking at the Lyt 1 helper cell level. If alloantigenic differences exist both with regard to SD and non-MHC, then there will be a normal generation of killer T cells against SD determinants in the presence of AFP [361]. These findings indicate that lymphocyte activating, non-MHC determinants can circumvent the mechanism of AFP-induced inhibition, presumably by supplying a non suppressible helper T cell signal to the 'poised CTL' reacting against SD. This contention is supported by the findings of *Schendel and Bach* [416] which indicate that lymphocyte activation by non-MHC determinants can indeed provide the helper T cell signal to CTL precursor cells.

It is clear from the observations outlined above that AFP will not serve as a general inhibitory agent for all immunological reactions. On the contrary, AFP appears to regulate TD antibody synthesis and certain cell-mediated reactions in a highly selective manner. A primary target for AFP-mediated suppression would appear to be an Lyt 1⁺ T helper cell required for

antibody responses to TD antigens and for LD-SD collaboration with pre-killer cells in the cellular events leading to optimal CTL generation [15, 28]. Data obtained by other workers failing to obtain significant inhibition of certain murine [190, 423] or rat [355, 421] immune reactions in the presence of AFP should be more easily understood when the above knowledge as to cellular and genetic restrictions is taken into account.

There have been a number of reports demonstrating that human AFP is capable of suppressing certain types of lymphocyte functions in vitro [25, 175, 251, 253, 321, 501]. *Yachnin and Lester* [501] have shown that AFP isolated from human fetal liver is a more potent suppressor of mitogen- and alloantigen-induced lymphocyte transformation by 1-3 orders of magnitude over tumor-derived AFP. Subsequent studies by this group [251] showed a correlation between the relative amount of a particular negatively charged molecular variant of AFP and immunosuppressive strength. Thus, fetal liver derived AFP, which has a high content of the electronegative species, is strongly immunosuppressive, while the usually weaker inhibitory properties of various tumor AFP isolates can be linked to the relative amounts of the negatively charged molecular variant they contain [251]. In view of the highly selective nature of the immunosuppressive action of murine AFP, we undertook an investigation to determine whether human AFP might also exert selective inhibitory effects on B and T cell proliferative responses in vitro [321]. The effects of human fetal-derived AFP were compared on lymphocyte activation induced by the human B cell mitogen protein A of *Staphylococcus aureus* strain Cowan I [144], by the T cell mitogen PHA, and by irradiated allogeneic lymphocytes in the one-way mixed lymphocyte reaction. Mitogenic responses to PHA were strongly inhibited in a dose-dependent manner over an AFP concentration range of 300-18 $\mu\text{g/ml}$. Importantly, PHA-reactive T cells were shown to be inhibited by a mechanism other than binding interference or competition with mitogen for cell surface receptors. In parallel cultures the proliferative response to protein A expressing *S. aureus* was normal or slightly enhanced in the presence of AFP. Strong suppression of the MLR generally requires higher concentrations of AFP than were necessary for inhibiting PHA responses [321]. The relative insensitivity of human MLR to AFP-mediated suppression, in comparison to PHA responses, may be an indication that, as in the murine system, human AFP is capable of exerting selective inhibitory effects on certain T cell subclasses and/or that the inhibitory action of human AFP on allogeneic reactions is restricted by the genetic relationship between responding and stimulating cells. Evidence does exist for functionally distinct subpopulations of human

T cells [86], and several reports indicate that mitogens and alloantigens may activate separate T cell subpopulations [18, 454]. Studies by *Gupta and Good* [175] and *Littman et al.* [259] would also suggest that human AFP is selective in its suppressive effects on in vitro cellular immune responses. Moreover, there are reports of AFP-mediated augmentation of mitogen and alloantigen-stimulated lymphocyte proliferation [85, 321, 441]. We consider that the immunoenhancing effects of AFP may be a physiologically relevant phenomenon. A growth-promoting effect by AFP on distinct cell types would be consistent with its frequent association with rapidly dividing cell populations in the fetus and newborn, and may to some extent reflect a shared property with albumin, a closely related protein thought to be essential for growth of mammalian lymphocytes in vitro. It should also be noted that unlike our experience with murine AFP we [321] and others [501] have observed a wide variation in the suppressive effects among individual isolates of human AFP. The exact reasons for these variations are not clear, but differences in methodology employed to isolate [64] and test [321] the proteins, the fetal versus tumor origin of the protein [501], the existence of a unique immunosuppressive subspecies [251] which may undergo postsynthetic modification [252], and the loss of active moieties during purification which are normally bound to AFP in the native state [226] are all possible contributing factors. Only further detailed investigations will determine which, if any, of these variable factors can satisfactorily explain presumed inconsistencies in the literature. Nevertheless, on the basis of the large body of information presently available, it is possible to conclude with a high degree of certainty that some molecular species of AFP do indeed possess an intrinsic capacity of mediate highly selective and powerful immunoregulatory influences on immune systems in vitro. AFP molecules with intrinsic suppressive properties may, however, also act in conjunction with other active factors in complicated immunoregulatory pathways.

We have studied the mechanism of AFP-mediated suppression on in vitro antibody synthesis in the murine system [167, 322]. Experiments were performed to determine whether appropriate in vitro exposure of normal adult spleen cells to AFP would result in the activation of a population of 'suppressor cells'. In initial studies adult spleen cells were cultured in Marbrook chambers [266] in the presence of AFP or normal mouse serum (NMS) at a final concentration of 200 $\mu\text{g/ml}$ for 4 days. The precultured cells were then washed and 1×10^6 cells were transferred to secondary assay cultures consisting of 20×10^6 fresh normal syngeneic spleen cells along with optimal immunizing doses of TD antigen. After an additional 4 days of

incubation the assay cultures were harvested and IgM antibody synthesis was measured. Spleen cells precultured in AFP were shown to effectively suppress primary IgM antibody responses, while cells precultured in NMS did not [322]. The fact that suppressor activity was significantly enriched in precultures of purified splenic T cells in comparison to whole spleen precultures, and that suppressor activity was absent in precultures of nude mouse spleen [322] indicated that the AFP-induced inhibitory cells were T lymphocytes. Interestingly, these adult AFP-activated inhibitory T cells showed many similarities with the previously mentioned naturally occurring newborn suppressors (table I) in terms of cell type, function, and efficiency of suppression. Like the newborn inhibitory T cells, adult AFP-activated T lymphocytes were found to suppress TD, but not TI antibody synthesis [318, 322].

Dilution analysis showed that as few as 10^4 adult inhibitory T cells, representing 0.05% of the total assay culture cell population markedly inhibited anti-SRBC responses [322]. In view of the functional similarities between newborn and adult inhibitory T cells we sought to determine whether the two cell populations might have common cell surface differentiation antigen phenotypes. Table VIII shows the effects of cytotoxic pretreatment of AFP-activated adult inhibitory T cells with anti-T cell reagents on their subsequent ability to inhibit TD antibody synthesis in secondary assay cultures. Treatment with heterologous anti-T or anti-Thy-1 antibodies and complement eliminates the ability to initiate suppression. Likewise, cytotoxic pretreatment with anti-Lyt 1 antibody abolished the suppressing activity, while anti-Lyt 2 failed to impair the inhibitory effect. These results therefore show that, like naturally occurring newborn suppressor (table II), the AFP-induced inhibitory cells in adult spleens also belong to the Lyt 1+2- subset of T lymphocytes. Additional studies were performed to determine the cell surface Ia antigen phenotype of adult AFP-activated inhibitory T cells [200]. The results of a typical experiment are presented in table IX. It can be seen that the suppressor activity was abrogated by cytotoxic pretreatment with anti-H-2^k, anti-Ia^k, anti-I-ABJ^k and anti-I-J^k antisera, but not by anti-I-EC^k. Only antisera which contained anti-I-J^k reactivity were effective in removing the suppressing ability thereby adding I-J coded determinants to the already known existence of Lyt 1 determinants on adult AFP-activated inhibitory cells. Thus, adult splenic T cells activated by a 4-day preculture in the presence of AFP could be shown to display the very same functional properties and cell surface antigen phenotype (Thy-1⁺, Lyt 1+2⁻, I-J⁺) as the naturally occurring newborn suppressing cells. Moreover, the rate of dis-

Table VIII. Cell surface Lyt differentiation antigen phenotype of AFP-induced adult inhibitory T cells

Preculture	Cytotoxic treatment with antisera and complement	Assay culture, % of control PFC response (mean \pm SEM of 5 experiments)
-	-	104 \pm 4
TNMS	-	49 \pm 3
TAFP	-	50 \pm 2
TAFP	+ RC	92 \pm 5
TAFP	anti-T + RC	84 \pm 4
TAFP	anti-Thy-1.2 + RC	90 \pm 4
TAFP	anti-Ly 1 + RC	47 \pm 3
TAFP	anti-Ly 2 + RC	

20×10^6 Ig-anti-Ig column purified adult CBA/J spleen T cells were precultured for 4 days in Marbrook chambers in the presence of 200 μ g/ml normal mouse serum (NMS) or alpha-fetoprotein (AFP) [see references 318 and 322 for details]. Precultured T cells were washed, treated with antisera plus rabbit complement (RC), and aliquotes equivalent to 10^6 control untreated cells were tested for inhibitory activity on IgM anti-SRBC PFC responses in secondary assay cultures. The number of day-4 IgM anti-SRBC PFC in control cultures without added precultured T cells was 832 ± 57 .

appearance of inhibitory T cells from the spleens of newborn mice with increasing age is closely paralleled by a decrease in the levels of serum AFP [318]. On the basis of these findings we would conclude that endogenous AFP may play an important immunoregulatory role in the developing immune system of the fetus and newborn via the induction of such inhibitory T lymphocytes. In support of this contention are reports that both newborn and adult AFP-activated inhibitory cells can function in vivo to suppress IgG and IgA antibody synthesis [200], and that adult mice injected with AFP have heightened susceptibility to Moloney sarcoma virus oncogenesis due to AFP-induced suppressing T lymphocytes [157]. In the human system we [322] and others [14] have shown that purified human AFP can activate in vitro cultured T cells isolated from normal peripheral blood to become inhibitors of mixed lymphocyte reactivity. The results presented in table X demonstrate that normal peripheral blood T lymphocytes exposed in vitro to purified fetal-derived AFP for 4 days can also effectively suppress PHA-stimulated lymphocyte transformation. The controls for this experiment consisting of T cells precultured in an equivalent concentration of human fetal-derived albumin failed to induce any inhibitory activity for mitogen

Table IX. Presence of I-J determinants on AFP-induced adult CBA/J inhibitory T cells

Preculture	Treatment	H-2 region detected								Assay culture
		K	I-A	I-B	I-J	I-E	I-C	S	G	
	antiserum + RC									I _g M SRBC PFC/ culture \pm SEM (% suppression)
-	-									668 \pm 99
TNMS	-									716 \pm 96(0)
T _{AFP}	-									324 \pm 36(51)
T _{AFP}	RC									234 \pm 57(65)
T _{AFP}	anti-T									652 \pm 41(2)
T _{AFP}	anti-Thy 1									656 \pm 81(2)
T _{AFP}	C3H. SW anti-C3H	K	K	K	K	K	K	K	K	636 \pm 20(5)
T _{AFP}	A. TH anti-A. TL	K	K	K	K	K	K	K	K	680 \pm 62(0)
T _{AFP}	(A. THXBIO. HTT) F ₁ anti-A. TL	K	K	K	K	K	K	K	K	764 \pm 70(0)
T _{AFP}	BIO. A (3R) anti-BIO. A (5R)				K	K	K	K	K	868 \pm 62(0)
T _{AFP}	BIO. S (7R) anti-BIO. HTT					K	K	K	K	277 \pm 24(58)

Adult AFP-activated inhibitory T cells (T_{AFP}) were generated in vitro according to procedures described in detail elsewhere [see table VIII, and references 318 and 322]. The Ia phenotype of T_{AFP} was determined as described for newborn inhibitory T cells in table III.

Table X. Human AFP-induced suppressor T cells: demonstration on PHA response

Preculture		³ H-TdR incorporation, cmp (mean ± SE)	
T cells precultured in	number of precultured cells added to assay culture	assay culture PHA 1.0 µg/ml	assay culture PHA 0.1 µg/ml
—	—	57,354 ± 1,259	26,321 ± 2,031
Albumin	10 ⁵	51,540 ± 2,468	20,475 ± 976
AFP	10 ⁵	37,027 ± 2,930	11,825 ± 1,454
Albumin	10 ⁴	58,164 ± 1,115	21,065 ± 1,025
AFP	10 ⁴	39,320 ± 1,544	10,667 ± 1,804

Normal human peripheral blood T lymphocytes, isolated on Ig-anti-Ig columns, were pre-cultured at 8×10^5 per ml in Marbrook chambers for 4 days in the presence of purified human fetal-derived AFP or albumin at final concentrations of 200 µg/ml. 1×10^4 pre-cultured T cells were transferred to assay cultures consisting of 2×10^5 syngeneic peripheral blood lymphocytes and PHA. Mitogen responses were measured at 72 h of culture.

responses. Indeed, we have consistently observed that purified human fetal albumin lacks suppressive activity when added directly to mitogen- and allo-antigen-stimulated lymphocyte cultures [321], or to precultures to test for induction of inhibitory cells for MLR [311]. On the basis of the above-mentioned findings and our previously discussed data in the murine system, one could speculate that the AFP present in cord blood may function as an inducer of the human newborn suppressor T cells described by *Olding et al.* [341–344].

In addition to the indirect mode of immunosuppressive action by AFP via the induction of regulatory suppressor cells, we have obtained evidence which suggests that AFP can also exert direct antiproliferative effects on select populations of MLC-reactive T cells [359]. Thus, in MLR studies we [359] have shown that addition of murine AFP to isolated anti-Ia T blasts responding in secondary MLC results in an almost immediate block of ³H-thymidine incorporation following restimulation. The question of whether this effect is related to the cell-binding properties of AFP [33, 109, 462] remains to be determined. Extensive studies in both the murine and human systems would therefore indicate that AFP, in its purified form, will suppress either directly or indirectly certain T cell-mediated immune reactions while leaving others unaffected or sometimes enhanced. Existing evi-

dence suggests that AFP does not act to affect B cell functions in a direct manner. However, there is evidence that AFP can exert suppressive effects on other cell types, including macrophages [207, 346], and estrogen-sensitive tumor cells [440] implying a more general biological significance for the regulatory properties of AFP. A recent series of findings indicate that AFP may be also important in the regulation of autoimmune responses. Human AFP has been reported to inhibit the binding of myasthenia gravis antibody to acetylcholine receptor antigen in vitro [5, 56]. Interestingly, the authors suggest that the existence of AFP in the fetus and newborn may explain the observation that transitory neonatal myasthenia gravis occurs only in a minority of cases in spite of the presence of transferred maternal anti-acetylcholine receptor antibodies. Additional preliminary reports suggest that the development of experimental autoimmune myasthenia gravis [55] and allergic encephalomyelitis (EAE) [4] can be prevented in animals by appropriate pretreatment with injections of AFP. In a separate study [147] it was noted, however, that while newborn rats with high endogenous levels of AFP do have a low incidence of EAE, a close correlation between decreasing serum levels of AFP and increased susceptibility to EAE is not observed. *Fujinami et al.* [147] therefore concluded that the presence of AFP does not explain the lack of disease in newborn rats. Finally, there is strong evidence emerging in the murine system which supports the contention that AFP may play a very important role in the prevention of autosensitization [*Hooper and Murgita*, 'Alpha-fetoprotein suppresses autoreactive T cell proliferation in vitro', submitted for publication]. In this study we have shown that neonatal or adult murine Lyt 1⁺ T cells reacting in autologous mixed lymphocyte reactions are suppressed in a highly manner by purified fetal-derived AFP in concentrations that are well below the normal endogenous serum levels in the fetus and newborn. As we have pointed out earlier [311], the possible role of immunoregulatory AFP in the clinical course of autoimmune disease is not yet clear. Nevertheless, the above-mentioned findings with their obvious potential theoretical and clinical importance should serve to stimulate further investigations in this area.

C. Regulation of Natural Killer Cell Activity

Natural killer (NK) cells constitute a distinct group of cells not belonging to any of the 'classical' types of cells in the immune system [194, 231]. They have recently attracted great interest due to their lytic effect on certain malignant cells in vitro. There is also a positive correlation between the level of NK activity in the murine system and the in vivo resistance to certain

tumor types [229]. In addition to their capacity to kill tumor cells, NK cells have been found to have lytic reactivity towards certain bone marrow stem cells [Hansson et al. 185a] as well as against immature, cortical thymocytes [184]. NK cells would therefore seem to be endowed with a special ability to kill certain cells expressing embryonic or 'primitive' features [449]. As most targets for NK cells include stem cells in the bone marrow as well as immature thymocytes it is likely that natural killer cells would also express some important features in relation to the neonatal period. Since this is clearly true for the rodents we include here a summary of the results of NK activity in relation to age.

A characteristic feature of the NK system in mice and rats is the rapid rise and fall in NK activity with age [193, 230]. Thus, NK reactivity is largely absent in fetal liver cells and in the spleens of newborn mice but will show a rapid rate of appearance at around 3 weeks of age. Activity will peak at 6-8 weeks of age and will then decline. A similar although not as abrupt pattern is displayed in the rat. Human NK cell activity shows a less impressive correlation to age although an age impact is clearly noted [67]. There may be several underlying reasons why NK cells seem to be comparatively more completely 'suppressed' in the newborn mouse than several other cellular types. One possibility would be that these cells are physically absent in the very young animals. In support of such a view are the findings that spleen cells from young mice (2 weeks old) possess only about half the number of NK cells as measured in a target-binding assay [Roder, personal commun.] as are in young adult mice. However, considering the relatively high amounts of erythropoietic cells in newborn spleen compared to adult [492], dilution effects must seriously be considered. Another plausible reason for the low level of NK activity noted in newborn mice may reside at the level of regulatory 'suppressing' cells. Thus, it has been known for some time in adult mice that the organ distribution of NK activity is inversely related to the actual presence of 'natural' target for NK activities in the same organ [229]. Thus, thymus and bone marrow which constitute two organ systems quite low in NK activity are also the two organs with the highest numbers of cells that can be lysed *in vitro* by NK cells [184, 185]. The frequency of such naturally occurring NK targets is significantly increased in the fetal and newborn periods both in murine and human systems. These target cells can be shown to be able to function as efficient cold target inhibitors in NK cytolytic assays [184, 185]. It would therefore follow that the presence of such potential target cells in a given organ would serve to reduce (i.e. suppress) the

actual available NK cells in the same population. As this 'cold target' inhibition would be expected to also involve conjugate formation between NK cells and target cells in vivo this would automatically lower the number of NK cells available for the conventional target conjugate assay in vitro and thus yield false low values. Finally, a third possible reason why newborn mice have close to undetectable levels of NK cells may be at the level of endogenous regulation of interferon levels [284, 422]. The newborn mouse does seem to have somewhat altered macrophage activities [260]. Polyinosinic-polycytidylic acid (poly I:C), an agent known to function via macrophages to induce NK cells through interferon release has been found by us to be an inefficient inducer when using spleen cells from newborn mice [349, 350]. On the other hand, the direct addition of interferon to newborn mouse spleen cells could induce a low but significant degree of NK activity. As interferon is known to be the major regulator of NK activity [158, 159], we consider it likely that the newborn mouse may be particularly deficient with regard to macrophage-mediated interferon production. If this mode of interferon release is an important factor in maintaining normal levels of NK activity (for which there is some precedent) [94] this may well add to the inefficient milieu with regard to the creation of sizable numbers of functioning NK cells in newborn mice. It is interesting to note that serum from newborn, but not adult mice inhibit in vitro NK activity [77]. Our findings suggest that fetal-derived AFP may in this regard play a role as poly I-C will fail to induce efficient NK activation in adult spleen cells if AFP is present in physiological amounts [349, 350]. Trivial reasons for this suppressing activity of AFP could be excluded and interferon added to adult spleen cells in the presence of AFP did yield, as expected, a significant increase in NK activity [350a].

In summary, we would thus conclude that the very low NK activities observed particularly in newborn rodents can be explained as a result of several factors all interacting to yield an 'NK-suppressing' milieu. As NK cells would seem to be able to kill certain stem cells in the bone marrow one could argue that such low NK activity at this stage may have some beneficial values for the individual.

VI. Pregnancy-Associated Immunoregulatory Activity

A most remarkable feature of mammalian pregnancy is the existence of what appear to be several precisely coordinated physiological processes

which together account for the protection of the histoincompatible fetus from maternal immune attack. Thus, attempts to define the mechanisms responsible for the successful maternal-fetal immunological co-existence have led to a number of concepts including (a) effective cellular and non-cellular anatomical separations at the maternal-fetal interphase; (b) hypo-antigenicity of the fetal-placental unit; (c) interruption of potentially harmful maternal antifetal cell-mediated immune responses via specific blocking factors (i. e. antibody-mediated tolerance-enhancement), and (d) transient hyporeactivity of the maternal immune system. Accumulated experimental evidence suggests that each of these processes may be playing a contributory role [17, 37-40, 107, 126, 191, 399]. However, while there does seem to be general agreement that exemption of the fetus from damaging maternal immune reactions is at least in part the result of the combined effects of several processes, there is no consensus as to the relative importance of each in maintaining this delicately balanced immunological liaison. A general discussion of the complex immunobiology of the maternal-fetal relationship can be found in recent review articles [37, 40, 399]. We will confine our remarks here to include only a brief discussion on the status of the maternal immune system during pregnancy. The question of whether immune functions remain unchanged, or become hypo- or hyperreactive during pregnancy is, within the context of the present article, to be considered quite relevant. For example, as pointed out by *Beer et al.* [39] one cannot completely disassociate the developing immune system of the fetus and newborn from that of the mother since there is known to be a significant level of maternal-to-perinatal transfer of serum proteins (including immunoglobulins) and milk leukocytes. Such an exchange of active humoral and cellular components is likely to have profound effects on the ontogenetic development of immune reactivity in the fetus and newborn.

There is now abundant evidence to show that the pregnant female normally does recognize and react against fetal antigens [37]. The failure of such sensitization to prejudice the fetus has been attributed to the presence of specific serum-blocking factors (thought to be antibodies and/or antigen-antibody complexes) which can be shown to effectively prevent the expression of maternal cell-mediated immunity *in vitro* [192, 502]. According to this premise some form of active response by the pregnant female toward the fetus would therefore be advantageous, if not essential, to the survival of the fetal 'allograft'. However, it is also quite possible that certain selective depressions of maternal immune reactivity should have to occur during pregnancy representing an additional beneficial adaptive response to protect

the fetus. Such alterations in the immune responsiveness of the pregnant female could conceivably exist at several different levels. There is, for example, evidence that certain maternal lymphocyte functions may be suppressed at intrinsic [45, 203, 265, 289, 384], extrinsic [47, 189, 219, 248, 357], local [19, 35, 146, 428] and systemic [16] levels. Pregnancy-associated immunosuppression is reported to be both specific [51, 195, 436, 437, 473] and nonspecific [30, 44, 179, 274-276], and to be mediated by humoral factors [332, 418, 452, 453], and by cells [46, 83, 84, 93, 463].

A number of physiological changes associated with the immune system are known to occur during pregnancy. It seems quite well established, for example, that the thymus undergoes transient involution during pregnancy in mice, rats, and humans [30, 95, 399]. There are also reports of splenic hyperplasia [10] and increased numbers of T cells in the lymph nodes draining the uterus [19, 201]. Information concerning pregnancy-associated changes in circulating lymphocyte populations appears somewhat controversial. Thus, there are reports suggesting that the percentage and absolute numbers of T cells are decreased during pregnancy [60], that B cell numbers decline while T cells remain unchanged [91], that the percentage of T cells temporarily drops together with an increase in B lymphocytes [459], and that the frequencies of B and T cells do not vary significantly throughout pregnancy [31]. It may be possible to reconcile some of these findings by considering that subtle redistributions in lymphocyte subpopulations are continuously occurring at various stages of pregnancy perhaps in response to changing hormonal levels [91, 415].

Evidence that immune reactivity is lowered during pregnancy includes altered survival of allografts [195, 279, 399], and suppression of mitogen responses [16, 35, 146, 203, 384], cell-mediated cytotoxicity [437, 473], NK activity [29], host versus graft reactions [93], antibody synthesis [30, 283, 313], and macrophage function [364]. Systemic suppression appears to be weak [16] and some immune functions are clearly not intrinsically inhibited at all during the gestational period [78, 79]. Circulating pregnancy-associated humoral factors may therefore be playing important extrinsic immunoregulatory roles by providing effective local immunosuppression. A number of studies have documented the *in vitro* immunosuppressive effects of pregnancy sera [139, 219, 220, 248]. Attempts to isolate and characterize the active factors in sera collected at different stages of pregnancy have led to varying degrees of success. Pregnancy-associated alpha-globulins have been shown to inhibit a variety of *in vitro* lymphocyte functions, including mitogen- and alloantigen-induced T cell proliferation [418, 452,

453]. Interestingly, *Stimson* [452] has shown that a high molecular weight glycoprotein isolated from pregnancy serum called alpha-macroglobulin shows some degree of selectivity in its in vitro immunosuppressive action. Thus, T cell stimulation induced by Con A, PHA, allogeneic cells, and tuberculin was found to be effectively blocked by physiological concentrations of alpha-macroglobulin. On the other hand, B cell proliferation induced by LPS and by anti-F(ab)₂ serum was shown to be less affected by the pregnancy-associated alpha-globulin suggesting that T cells are a primary target in this inhibitory pathway. This premise was supported by a subsequent study showing that alpha-macroglobulin binds to subpopulations of T lymphocytes and monocytes, but not to B cells [451]. The possible role of immunosuppressive AFP molecules in pregnancy sera has also been investigated [139, 313, 474]. A study in the murine system has shown that there are selective depressions in certain immune functions during pregnancy which may in part be attributable to circulating levels of AFP [313]. Pregnancy induced inhibition of in vitro and in vivo specific antibody synthesis was most pronounced on the IgA and IgG classes with little or no significant effect on IgM antibody production observable in vivo [131]. It is notable here that this same pattern of selective inhibition of antibody production was previously seen in mice receiving injections from birth through young adulthood with homologous amniotic fluid [337] and in studies on the effect of purified fetal-derived AFP on primary and secondary antibody responses in vitro [315]. Spleen cells from mid-term pregnant mice had suppressed mitogenic responses to PHA, a slightly reduced response to Con A and a normal response to LPS. Allogeneic reactivity of splenic T lymphocytes from pregnant animals was significantly enhanced over non-pregnant controls. However, pregnancy sera were effective in blocking in vitro TD antibody synthesis as well as T mitogen and alloantigen-induced lymphocyte proliferation. Much of this inhibitory effect could be abrogated by selective removal of AFP on antibody-agarose affinity columns. Finally, normal mouse serum became similarly immunosuppressive in vitro when purified AFP of fetal origin was added to it in concentrations approximating the levels present in the sera of mid-term pregnant mice [313]. Studies by *Toder et al.* [474] suggest that AFP-mediated immunosuppression can be observed even in the early stages of murine pregnancy if the circulating levels of AFP are artificially boosted by injections of purified AFP. In addition, correlative studies in pregnant women between lymphocyte functions and serum AFP levels are compatible with the idea that AFP may be one among a number of factors contributing to pregnancy-induced immunosuppression [139].

Immunoprotection of the fetus could also be provided in part by the regulatory influences of the endocrine milieu of pregnancy [10]. There have been reports which suggest that the human chorionic gonadotropin (HCG) hormone which is synthesized early in pregnancy by the trophoblast, may have immunosuppressive properties [6, 103, 373]. However, it is not clear from subsequent studies whether the inhibitory activity found associated with commercially prepared HCG extracts from pregnancy urine was actually due to the hormone or to a co-purified immunoregulatory contaminating substance(s) [296, 309]. There would seem to be more substantial evidence that some of the steroid hormones may possess physiologically relevant immunosuppressive activity [3, 393, 499]. Studies by *Siiteri et al.* [428] indicate, for example, that effective local concentrations of progesterone may exert significant suppressive effects on maternal T cell-mediated immune functions. Cortisol was also found to be an effective inhibitor of human MLR, although *Kasakura* [220] has shown that the inhibitory activity in pregnancy sera does not correlate with serum cortisol levels.

There is now a growing opinion that certain gestational hormones as well as some other pregnancy-associated substances, are most likely able to exert strong local immune suppression rather than mediating broad systemic immunoprotection. It is possible that the various active pregnancy-associated factors which have been described act in concert to impart beneficial immunoregulatory effects at the maternal-fetal interphase. An immunoregulatory role for pregnancy plasma factors may also be indicated by studies demonstrating the abortagenic and immunotherapeutic effects of antibodies directed against such substances [285, 286, 433]. The mechanism of suppression by pregnancy related factors could involve direct inhibitory action on relevant target lymphocyte functions and/or an indirect regulatory effect via the induction of suppressor cells. Indeed, there is now some evidence to suggest that specific [83, 84, 93] and nonspecific [46, 463] inhibitory cells do exist during pregnancy.

VII. General Conclusions

The immune response at the cellular level represents an intricate system of inductive and regulatory cell circuits. An analysis of the ontogeny of the various subsets of cells which establish the system indicate a precisely coordinated sequence of reaction patterns to achieve an optimal situation at any given time during development. The developing mammalian fetus

must have available at and before birth a immune system functioning sufficiently to protect against invading microorganisms as well as against maternal lymphocytes sensitized against paternal alloantigens. At the same time there must be appropriate controls to prevent potentially damaging autoreactive processes. The picture that emerges from studies of the developing fetus and newborn strongly indicate the existence of reactivity patterns designed to deal with these requirements. There clearly does exist a gradual development of immune capacity in several cell lineages during gestation. This is followed by some quite dramatic changes in reactivity profiles subsequent to delivery. The maturation of the immune system after birth can be shown in many cases to closely parallel the normal physiological decay of strong elements of regulatory suppressor cells and inhibitory humoral factors. The fetus and newborn can thus be shown to contain humoral immunosuppressive substances of both endogenous and exogenous origin, as well as efficient suppressor-inducing cellular systems with the potential to interrupt invading aggressive T lymphocytes. It is likely that deeper understandings of how these naturally occurring suppressing factors and cells are induced into such effective functions may lead to positive applications in certain clinical situations where selective immune regulation is warranted.

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IX. References

- 1 Abelev, G.I.: Alphafetoprotein in ontogenesis and its association with malignant tumors. *Adv. Cancer Res.* 14: 295 (1971).
- 2 Abeny, E.R.; Cooper, M.D.; Kearney, J.F.; Lawton, A.R.; Parkhouse, R.M.E.: Sequential expression of immunoglobulins on developing mouse B lymphocytes. A systematic survey that suggests a model for the generation of immunoglobulin isotype diversity. *J. Immun.* 120: 2041 (1978).
- 3 Ablin, R.J.; Bruns, G.R.; Guinan, P.; Bush, I.M.: The effect of estrogen on the incorporation of ³H-thymidine by PHA-stimulated human peripheral blood lymphocytes. *J. Immun.* 113: 705 (1974).
- 4 Abramsky, O.; Soffer, D.; Brenner, T.: Prevention and treatment of experimental

- allergic encephalomyelitis by alpha-fetoprotein. *Neurology, Minneap.* 30: 446 (1980).
- 5 Abramsky, O.; Lisak, R.P.; Brenner, T.; Zeidman, A.; Beyth, Y.: Significance in neonatal myasthenia gravis of inhibitory effect of amniotic fluid on binding of antibodies to acetylcholin receptor. *Lancet ii*: 1333 (1979).
 - 6 Adcock, E.W., III; Teasdale, F.; August, C.S.; Cox, S.; Meschia, G.; Battaglia, F.C.; Naughton, M.A.: Human chorionic gonadotropin. Its possible role in maternal lymphocyte suppression. *Science* 181: 845 (1973).
 - 7 Adinolfi, A.; Adinolfi, M.; Lessof, M.H.: Alpha-fetoprotein during development and in disease. *J.med.Genet.* 12: 138 (1975).
 - 8 Adler, W.H.; Takiguchi, T.; Marsh, B.; Smith, R.T.: Cellular recognition by mouse lymphocytes in vitro. I. Definition of a new technique and results of stimulation by phytohemagglutinin and specific antigens. *J.exp.Med.* 131: 1049 (1970).
 - 9 Adler, W.H.; Takiguchi, T.; Smith, R.T.: Effect of age upon primary alloantigen recognition by mouse spleen cells. *J.Immun.* 107: 1357 (1971).
 - 10 Ahlquist, F.: Endocrine influences on lymphatic organs, immune responses, inflammation and autoimmunity (Almquist & Wiksell, Stockholm 1976).
 - 11 Ahmed, A.; Scher, I.; Sharrow, S.O.; Smith, A.H.; Paul, W.E.; Sahcs, D.H.; Sell, K.W.: B lymphocyte heterogeneity. Development and characterization of an allo-antiserum which distinguishes B lymphocyte differentiation alloantigens. *J.exp.Med.* 145: 101 (1977).
 - 12 Ahmed, A.; Scher, I.; Sell, K.W.: Functional studies on the ontogeny of the M-locus product. A surface antigen of murine B lymphocytes; in Eisvoogel, Roos, Zerkle-maker, Leucocyte membrane determinants regulating immune reactivity, p.703 (Academic Press, New York 1975).
 - 13 Alpert, E.; Drysdale, J.W.; Isselbacher, K.J.; Schur, P.H.: Human alpha-feto-protein. Isolation, characterization and demonstration of microheterogeneity. *J.biol.Chem.* 247: 3792 (1972).
 - 14 Alpert, E.; Dienstag, J.L.; Sepersky, S.; Littman, B.; Rocklin, R.: Immunosuppressive characteristics of human AFP. Effect on tests of cell mediated immunity and induction of human suppressor cells. *Immunol. Commun.* 7: 163 (1978).
 - 15 Alter, B.J.; Bach, F.H.: Speculations on alternative pathways of T-lymphocyte response. *Scand.J.Immunol.* 10: 87 (1979).
 - 16 Anderson, D.J.: The responsiveness of various maternal mouse lymphocyte populations to mitogenic stimulations *in vitro*. *Cell.Immunol.* 41: 150 (1978).
 - 17 Anderson, J.M.: Transplantation? Nature's success. *Lancet ii*: 1077 (1971).
 - 18 Andersson, L.C.; Hayry, P.: Clonal isolation of alloantigen-reactive T cells and characterization of their memory functions. *Transplant.Rev.* 25: 121 (1975).
 - 19 Ansell, J.D.; McDougall, C.; Speedy, G.; Inchley, C.J.: Changes in lymphocyte accumulation and proliferation in the lymph nodes draining the pregnant uterus. *Clin.exp.Immunol.* 31: 397 (1978).
 - 20 Arai, S.; Yamane, I.; Tanno, Y.; Takishima, T.: Role of bovine serum albumin in blastoid transformation of lymphocytes by phytohemagglutinin. *Proc.Soc.exp.Biol.Med.* 154: 444 (1977).
 - 21 Argyris, B.F.: Role of macrophages in immunological maturation. *J.exp.Med.* 128: 459 (1968).
 - 22 Argyris, B.F.: Role of macrophages in the immune response; in Gottlieb, Developments in lymphoid cell biology, p.27 (CRC Press, Cleveland 1974).

- 23 Argyris, B. F.: Suppressor activity in the spleen of neonatal mice. *Cell. Immunol.* 36: 354 (1978).
- 24 Argyris, B. F.: Further studies on suppressor cell activity in the spleen of neonatal mice. *Cell. Immunol.* 48: 398 (1979).
- 25 Auer, I. O.; Kress, H. G.: Suppression of the primary cell-mediated immune response by human alpha-fetoprotein *in vitro*. *Cell. Immunol.* 30: 173 (1977).
- 26 Ayoub, J.; Kasakura, S.: *In vitro* response of foetal lymphocytes to PHA, and a plasma factor which suppresses the PHA response of adult lymphocytes. *Clin. exp. Immunol.* 8: 427 (1971).
- 27 Bach, F. H.; Bach, M. L.; Sondel, P. M.: Differential function of major histocompatibility complex antigens in T lymphocyte activation. *Nature, Lond.* 259: 273 (1976).
- 28 Bach, F. H.; Bach, M. L.; Sondel, P. M.: Differential function of major histocompatibility complex antigens in T-lymphocyte activation. *Nature, Lond.* 259: 273 (1976).
- 29 Baines, M. G.; Pross, H. F.; Miller, K. G.: Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IV. The suppressive effect of normal pregnancy. *Am. J. Obstet. Gynec.* 130: 741 (1978).
- 30 Baines, M. G.; Pross, H. F.; Millar, K. G.: Effects of pregnancy on the maternal lymphoid system in mice. *Obstet. Gynec., N. Y.* 50: 457 (1977).
- 31 Baines, M. G.; Pross, H. F.; Millar, K. G.: Lymphocyte populations in peripheral blood during normal human pregnancy. *Clin. exp. Immunol.* 28: 453 (1977).
- 32 Baker, P. J.; Reed, N. D.; Stashak, P. W.; Amsbaugh, D. F.; Prescott, B.: Regulation of the antibody response to type III pneumococcal polysaccharide. I. Nature of regulatory cells. *J. exp. Med.* 137: 1431 (1973).
- 33 Bankhurst, A. D.; Wittemeyer, S.; Eilliams, R. C., Jr.: A population of human cord blood mononuclear cells with surface alpha-fetoprotein. *Immunol. Commun.* 7: 187 (1978).
- 34 Bassett, M.; Coons, T. A.; Wallis, W.; Goldberg, E. H.; Williams, R. C.: Suppression of stimulation in mixed leucocyte culture by newborn splenic lymphocytes in the mouse. *J. Immun.* 119: 1855 (1977).
- 35 Bauminger, S.; Peleg, S.: Changes in immunological activity of rat lymphoid organs during pregnancy. *Clin. exp. Immunol.* 32: 179 (1978).
- 36 Bayard, B.; Kerckaert, J.-P.: Characterization and isolation of nine rat alpha-fetoprotein variants by gel electrophoresis and lectin affinity chromatography. *Biochem. biophys. Res. Commun.* 77: 489 (1977).
- 37 Beer, A. E.; Billingham, R. E.: Maternal immunological recognition mechanisms during pregnancy; in *Maternal recognition of pregnancy*. Ciba Found. Ser. 64: 293 (1979).
- 38 Beer, A. E.; Billingham, R. E.: Immunobiology of mammalian reproduction. *Adv. Immunol.* 14: 2 (1971).
- 39 Beer, A. E.; Billingham, R. E.; Head, J. R.; Parmely, M. J.: Possible influence of maternal-to-perinatal cell transfer on the development of host defenses; in Cooper, Dayton, *Development of host defenses*, p. 251 (Raven Press, New York 1977).
- 40 Beer, A. E.; Billingham, R. E.: Immunoregulatory aspects of pregnancy. *Fed. Proc.* 37: 2374 (1978).
- 41 Bendinelli, M.; Senesi, S.; Falcone, G.: Effect of adult peritoneal cells on the antibody response of newborn mice to sheep red blood cells. *J. Immun.* 106: 1681 (1971).

- 42 Bianco, C.; Nussenzweig, V.: Theta-bearing and complement receptor bearing lymphocytes are distinct populations of cells. *Science* 173: 154 (1971).
- 43 Birchner, H.; Glaser, M.; Herberman, R.B.: Suppression of cell-mediated tumour immunity by *Cornebacterium parvum*. *Nature, Lond.* 257: 396 (1975).
- 44 Birkeland, S.A.; Kristoffersen, K.: The fetus as an allograft. A longitudinal study of normal human pregnancies studied with mixed lymphocyte cultures between mother, father and mother-child. *Scand.J.Immunol.* 11: 311 (1980).
- 45 Birkeland, S.A.; Kristoffersen, K.: A longitudinal study of B lymphocyte response during normal human pregnancy. *J.Reprod.Fertil.* 2: 23 (1980).
- 46 Birkeland, S.A.; Kristoffersen, K.: Lymphocyte transformation with mitogens and antigens during normal human pregnancy. A longitudinal study. *Scand.J.Immunol.* 11: 321 (1980).
- 47 Bissenden, J.G.; Ling, N.R.; Mackintosh, P.: Suppression of mixed lymphocyte reactions by pregnancy serum. *Clin.exp.Immunol.* 39: 195 (1980).
- 48 Blaese, R.M.; Lawrence, E.C.: Development of macrophage function and the expression of immunocompetence; in Cooper, Dayton, Development of host defenses, p.201 (Raven Press, New York 1977).
- 49 Blomgren, H.; Andersson, B.: Evidence for a small pool of immunocompetent cells in the mouse thymus. *Expl. Cell Res.* 57: 185 (1969).
- 50 Blomgren, H.; Andersson, B.: Characteristics of the immunocompetent cells in the mouse thymus. Cell populations changes during cortisone-induced atrophy and subsequent regeneration. *Cell.Immunol.* 1: 545 (1971).
- 51 Bonnard, G.D.; Lemos, L.: The cellular immunity of mother versus child at delivery. Sensitization in unidirectional mixed lymphocyte culture and subsequent ⁵¹Cr-release cytotoxicity test. *Transplant.Proc.* 4: 177 (1972).
- 52 Borum, K.: Pattern of cell production and cell migration in mouse thymus studies by autoradiography. *Scand.J.Haematol.* 5: 339 (1968).
- 53 Bosing-Schneider, R.: Functional maturation of neonatal spleen cells. *Immunology* 36: 527 (1979).
- 54 Braciale, V.L.; Parish, C.R.: Inhibition of *in vitro* antibody synthesis by cyclophosphamide-induced suppressor cells. *Cell.Immunol.* 51: 1 (1980).
- 55 Brenner, T.; Abramsky, O.: Suppression of clinical and experimental myasthenia gravis by alpha-fetoprotein. *Neurology, Minneap.* 30: 380 (1980).
- 56 Brenner, T.; Beyth, Y.; Abramsky, O.: Inhibitory effect of alpha-fetoprotein on the binding of myasthenia gravis antibody to acetylcholine receptor. *Proc.natn.Acad. Sci. USA* 77: 3635.
- 57 Bufté, D.; Ribaut, C.: Immunosuppressive effect of a human hepatic glycoferroprotein, α -2-H-globulin. *Immunology* 29: 175 (1975).
- 58 Bullock, W.; Moller, E.: 'Spontaneous' B cell activation due to loss of normal mouse serum suppressor. *Eur.J.Immunol.* 2: 514 (1972).
- 59 Bullock, W.E.; Carlson, E.M.; Gershon, R.K.: The evolution of immunosuppressive cell population in experimental mycobacterial infection. *J.Immun.* 120: 1709 (1978).
- 60 Bulmer, R.; Hancock, K.W.: Depletion of circulating T lymphocytes in pregnancy. *Clin.exp.Immunol.* 28: 302 (1977).
- 61 Burakoff, S.J.; Finberg, R.; Glimcher, L.; Lemonnier, F.; Benacerraf, B.; Cantor,

- H.: The biologic significance of alloreactivity. The ontogeny of T-cell sets specific for alloantigens or modified self antigens. *J. exp. med.* 148: 1414 (1978).
- 62 Burns, F.D.; Marrack, P.C.; Kappler, J.W.; Janeway, C.A., Jr.: Functional heterogeneity among the T-derived lymphocytes of the mouse. IV. Nature of spontaneously induced suppressor cells. *J. Immunol.* 114: 1345 (1975).
- 63 Byrd, W.J.; Boehmer, H. von; Rouse, B.T.: The role of the thymus in maturational development of phytohemagglutinin and pokeweed mitogen responsiveness. *Cell. Immunol.* 6: 12 (1973).
- 64 Calderon, J.; Unanue, E.R.: Two biological activities regulatory cell proliferation found in cultures of peritoneal exudate cells. *Nature, Lond.* 253: 359 (1975).
- 65 Calkins, C.E.; Stutman, O.: Changes in suppressor mechanisms during postnatal development in mice. *J. exp. Med.* 147: 87 (1978).
- 66 Calkins, C.E.; Orbach-Arbouys, S.; Stutman, O.; Gershon, R.K.: Cell interactions in the suppression of *in vitro* antibody response. *J. exp. Med.* 143: 1421 (1976).
- 67 Campbell, A.C.; Waller, C.; Wood, J.; Aynsley-Green, A.; Yu, V.: Lymphocyte subpopulations in the blood of newborn infants. *Clin. exp. Immunol.* 18: 469 (1974).
- 68 Cantor, D.; Shen, F.W.; Boyse, E.A.: Separation of helper T cells from suppressor T cells expressing different Ly components. II. Activation by antigen: After immunization, antigen specific suppressor and helper activities are mediated by distinct T cell subclasses. *J. exp. Med.* 143: 1391 (1976).
- 69 Cantor, H.; Gershon, R.K.: Immunological circuits: cellular composition. *Fed. Proc.* 38: 2958 (1979).
- 70 Cantor, H.; Boyse, E.A.: Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses in a differentiative process independent of antigen. *J. exp. Med.* 141: 1376 (1975).
- 71 Cantor, H.; Asofsky, R.: Synergy among lymphoid cells mediating the graft-versus-host response. II. Synergy in GVH reactions produced by balb/c lymphoid cells of differing anatomic origin. *J. exp. Med.* 131: 235 (1970).
- 72 Cantor, H.; Boyse, E.A.: Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly+ cells in the generation of killer activity. *J. exp. Med.* 141: 1390 (1975).
- 73 Cantor, H.; Simpson, E.; Sats, V. L.; Fathmann, C. G.; Herzenberg, L. A.: Characterisation of subpopulations of T lymphocytes. I. Separation and functional studies of peripheral T-cell binding different amount of fluorescen anti-Thy 1.2 (Theta) antibody using a fluorescence-activated cell sorter (FACS). *Cell. Immunol.* 15: 180 (1975).
- 74 Cantor, H.; 55cVey-Boudrea, L.; Hugenberg, J.; Naidorf, K.; Shen, F.W.; Gershon, R.K.: Immunoregulatory circuits among T-cell sets. II. Physiologic role of feedback inhibition *in vivo*. Absence in NZB mice. *J. exp. Med.* 147: 1116 (1978).
- 75 Cantor, H.; Hugenberg, J.; McVay-Boudreau, L.; Eardley, D.D.; Kemp, J.; Shen, F.W.; Gershon, R.K.: Immunoregulatory circuits among T cell sets. Identification of a subpopulation of T helper cells that induces feedback inhibition. *J. exp. Med.* 148: 871 (1978).
- 76 Cantor, H.; McVay-Boudreau, L.; Hugenberg, J.; Naidorf, K.; Shen, F.W.; Gershon, R.K.: Immunoregulatory circuits among T cell sets. II. Physiologic role of feedback inhibition *in vivo*: Absence in NZB mice. *J. exp. Med.* 147: 1116 (1978).
- 77 Cantor, H.; Kasal, M.; Shen, F.W.; Leclerc, J.C.; Glimcher, L.: Immunogenetic analysis of 'natural killer' activity in the mouse. *Immunol. Rev.* 44: 3 (1979).

- 78 Carr, M.C.; Stites, D.P.; Fudenberg, H.H.: Cellular immune aspects of the human fetal maternal relationship. II. In vitro responses of gravida lymphocytes to phytohemagglutinin. *Cell.Immunol.* 8: 448 (1973).
- 79 Carr, M.C.; Stites, D.P.; Fudenberg, H.H.: Cellular immune aspects of the human fetal-maternal relationship. III. Mixed lymphocyte reactivity between related maternal and cord blood lymphocytes. *Cell.Immunol.* 11: 332 (1974).
- 80 Carter, B.G.; Rector, E.S.: The generation of antigen sensitive cells in the newborn mouse. *J.Immun.* 109: 1345 (1972).
- 81 Chakravarty, A.; Kubai, L.; Sidky, Y.; Auerback, R.: Ontogeny of thymus cell function; in Friedman, Thymus factors in immunity. *Ann.N.Y.Acad.Sci.* 249: 34 (1975).
- 82 Chanana, A.D.; Joel, D.D.; Schaedeli, J.; Hess, M.W.; Cottier, H.: Thymus cell migration. ³H-TdR-labeled and theta-positive cells in peripheral lymphoid tissues of newborn mice. *Adv.exp.Med.Biol.* 29: 79 (1972).
- 83 Chaouat, G.; Voisin, G.A.: Regulatory T cell subpopulations in pregnancy. I. Evidence for Suppressing Activity of the early phase of MLR. *J.Immun.* 122: 1383 (1979).
- 84 Chaouat, G.; Voisin, G.A.; Escalier, D.; Robert, P.: Facilitation reaction (enhancing antibodies and suppressor cells) and rejection reaction (sensitized cells) from the mother to the paternal antigens of the conceptus. *Clin.exp.Immunol.* 35: 13 (1979).
- 85 Charpentier, B.; Guttman, R.D.; Shuster, J.; Gold, P.: Augmentation of proliferation of human mixed lymphocyte culture by human alpha-fetoprotein. *J.Immun.* 119: 897 (1977).
- 86 Chess, L.; Schlossman, S.F.: Human lymphocyte subpopulations. *Adv.Immunol.* 25: 213 (1977).
- 87 Chisari, F.V.: Immunoregulatory properties of human plasma in very low density lipoproteins. *J.Immun.* 119: 2129 (1977).
- 88 Chiscon, M.O.; Golub, E.S.: Functional development of the interacting cells in the immune response. I. Development of T cell and B cell function. *J.Immun.* 108: 1379 (1971).
- 89 Chiu, K.M.; Faanes, R.B.; Choi, N.Y.: Activation of specific suppressor cells with heat-treated allogeneic tumor cells. *Cell.Immunol.* 49: 183 (1980).
- 90 Chiu, K.M.; Mortenson, R.F.; Osmand, A.P.; Gewurz, H.: Interactions of alpha-acid glycoprotein with the immune system. I. Purification and effects upon lymphocyte responsiveness. *Immunology* 32: 997 (1977).
- 91 Christiansen, J.S.; Andersen, A.R.; Osther, K.; Peitersen, B.; Bach-Mortensen, N.; Lebech, P.E.: The relationship between pregnancy, HCS and B lymphocytes. *Acta path.microbiol.scand.* 84: 313 (1976).
- 92 Chused, T.M.; Kassan, S.S.; Moser, D.E.: Macrophages requirement for the in vitro response to TNP Ficoll. A thymic independent antigen. *J.Immun.* 116: 1579 (1976).
- 93 Clark, D.A.; McDermott, M.R.: Impairment of host vs. graft reaction in pregnant mice. I. Suppression of cytotoxic T cell generation in lymph nodes draining the uterus. *J.Immun.* 121: 1389 (1978).
- 94 Clark, E.A.; Russell, P.H.; Egghart, M.; Horton, M.A.: Characteristics and genetic control of NK cell-mediated cytotoxicity activated by naturally acquired infection in the mouse. *Int.J.Cancer* 24: 688 (1979).
- 95 Clarke, A.G.: Pregnancy-induced involution of the thymus can be prevented by

- immunizing with paternal skin grafts: a strain-dependent effect. *Clin. exp. Immunol.* 35: 421 (1979).
- 96 Cline, M.J.: Ontogeny of mononuclear phagocytes; in Van Furth, Mononuclear phagocytes in immunity, infection, and pathology, p. 163 (Blackwell, Oxford 1973).
- 97 Cline, M.J.; Moore, M.A.S.: Embryonic origin of the mouse macrophage. *Blood* 39: 842 (1972).
- 98 Clough, J.D.; Mims, L.H.; Strober, W.: Deficient IgA antibody responses to arsanilic acid bovine serum albumin (BSA) in neonatally thymectomized rabbits. *J. Immun.* 106: 1624 (1971).
- 99 Cohen, J.J.; Claman, H.N.: Thymus-Marrow Immunocompetence. V. Hydrocortisone-resistant cells and processes in the hemolytic antibody response of mice. *J. exp. Med.* 133: 1026 (1971).
- 100 Cohen, L.; Howe, M.L.: Synergism between subpopulations of thymus-derived cells mediating the proliferative and effector phases of the mixed lymphocyte reaction. *Proc. natn. Acad. Sci. USA* 70: 2707 (1973).
- 101 Cohen, P.L.; Mosier, D.E.: Reactivity of steroid-resistant neonatal thymocytes. *Nature, Lond.* 251: 233 (1974).
- 102 Cohen, P.L.; Cross, S.S.; Mosier, D.E.: Immunological effects of neonatal infection with mouse thymic virus. *J. Immun.* 115: 796 (1975).
- 103 Contractor, S.F.; Davies, H.: Effect of human chorionic somatomammotrophin and human chorionic gonadotrophin on phytohaemagglutinin-induced lymphocyte transformation. *Nature new Biol.* 243: 284 (1973).
- 104 Cooperband, S.R.; Badger, A.M.: Suppressor factors in serum and plasma; in Neubauer, Naturally occurring biological immunosuppressive factors and their relationship to disease, p. 115 (CRC Press, Boca Raton 1979).
- 105 Cowing, C.; Pincus, S.H.; Sachs, D.H.; Dickker, H.B.: A subpopulation of adherent necessary cells bearing both I-A and I-E or C subregion antigens is required for antigen-specific murine T lymphocyte proliferation. *J. Immun.* 121: 1680 (1978).
- 106 Cowing, C.; Schwartz, B.D.; Dickler, H.B.: Macrophage Ia antigens. I. Macrophage populations differ in their expression of Ia antigens. *J. Immun.* 120: 378 (1978).
- 107 Currie, G.A.; Bagshawe, K.D.: The masking of antigens on trophoblast and cancer cells. *Lancet i*: 708 (1967).
- 108 D'Eustachio, P.; Edelman, G.M.: Frequency and avidity of specific antigen-binding cells in developing mice. *J. exp. Med.* 142: 1078 (1975).
- 109 Dattwyler, R.; Murgita, R.A.; Tomasi, T.B.: The binding of alpha-fetoprotein to murine T cells. *Nature, Lond.* 256: 656 (1975).
- 110 Davies, A.J.S.: The thymus and the cellular basis of immunity. *Transplant. Rev.* 1: 43 (1969).
- 111 Decker, J.M.; Sercarz, E.E.: Early simultaneous appearance of antigen binding cells in foetal sheep. *Nature, Lond.* 252: 416 (1974).
- 112 Dekruffy, R.H.; Kim, Y.T.; Siskind, G.W.; Weksler, M.E.: Age related changes in the *in vitro* immune response: Increased suppressor activity in immature and aged mice. *J. Immun.* 125: 142 (1980).
- 113 Dixon, F.J.; Weigle, W.O.: The nature of the immunologic inadequacy of neonatal rabbits by cell transfer studies. *J. exp. Med.* 105: 75 (1957).
- 114 Dixon, F.J.; Weigle, W.O.: The nature of the immunological inadequacy of neonatal

- rabbits. II. Antibody formation by neonatal spleen cells transferred to adult recipients. *J.exp.Med.* 110: 139 (1959).
- 115 Durandy, A.; Fischer, A.; Griscelli, C.: Active suppression of B lymphocyte maturation by two different newborn T lymphocyte subsets. *J.Immun.* 123: 2644 (1979).
- 116 Dutton, R.W.: Suppressor T cells. *Transplant.Rev.* 26: 39 (1975).
- 117 Duwe, A.K.; Singhal, S.K.: The immunoregulatory role of bone marrow. I. Suppression of the induction of antibody responses to T-dependent and T-independent antigens by cells in the bone marrow. *Cell.Immunol.* 43: 362 (1979).
- 118 Duwe, A.K.; Singhal, S.K.: The immunoregulatory role of bone marrow. II. Characteristics of a suppressor cell inhibiting the *in vitro* antibody response. *Cell Immunol.* 43: 372 (1979).
- 119 Dwyer, J.M.; Mackay, I.R.: The development of antigen-binding lymphocytes in foetal tissues. *Immunology* 23: 871 (1972).
- 120 Eardley, D.; Gershon, R.K.: Induction of specific suppressor T cells *in vitro*. *J.Immun.* 117: 313 (1976).
- 121 Eardley, D.D.; Staskawicz, M.O.; Gershon, R.K.: Suppressor cells. Dependence on assay conditions for functional activity. *J.exp.Med.* 143: 1211 (1976).
- 122 Eardley, D.D.; Gershon, R.K.: Feedback induction of suppressor T cell activity. *J.exp.Med.* 142: 524 (1975).
- 123 Eardley, D.D.; Hugenberg, J.; McVay-Boudreau, L.; Shen, F.W.; Gershon, R.K.; Cantor, H.: Immunoregulatory circuits among T cell sets. I. T helper cells induce other T cell sets to exert feedback inhibition. *J.exp.Med.* 147: 1106 (1979).
- 124 Eardley, D.D.; Shen, F.W.; Cantor, H.; Gershon, R.K.: Genetic control of immunoregulatory circuits. Genes linked to the Ig locus govern communication between regulatory T cell sets. *J.exp.Med.* 150: 44 (1979).
- 125 Edwards, G.E.; Miller, R.G.; Phillips, R.A.: Differentiation of rosette-forming cells from myeloid stem cells. *J.Immun.* 105: 719 (1970).
- 126 Edwards, R.G.; Coombs, R.R.A.: Immunological interactions between mother and fetus; in Gell, Coombs, Lachmann, *Clinical aspects of immunology*; 3rd ed., p.561 (Blackwell, Oxford 1975).
- 127 Eichmann, K.: Idiotype suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. *Eur.J.Immunol.* 5: 511 (1975).
- 128 Elgert, K.D.; Farrer, W.L.: Suppressor cell activity in tumor bearing mice. I. Dualistic inhibition by suppressor T lymphocytes and macrophages. *J.Immun.* 120: 1234 (1978).
- 129 Elliott, L.H.; Roszman, T.L.: Suppressor cell regulation of the spontaneously induced *in vitro* secondary antibody response. *Cell.Immunol.* 40: 46 (1978).
- 130 Erb, P.; Feldman, M.: The role of macrophages in the generation of T helper cells. II. The genetic control of the macrophage-T cell interaction for helper cell induction with soluble antigen. *J.exp.Med.* 142: 460 (1975).
- 131 Etlinger, H.M.; Chiller, J.M.: Suppression of immunological activities by mouse amniotic fluid. *Scand.J.Immunol.* 6: 1241 (1977).
- 132 Evans, M.M.; Williamson, W.G.; Irvine, W.J.: The appearance of immunological competence at an early age in New Zealand Black mice. *Clin.exp.Immunol.* 3: 375 (1968).
- 133 Feldman, M.; Beverley, P.C.L.; Woody, J.; McKenzie, I.F.C.: T-T interactions in the induction of suppressor and helper T cells: Analysis of membrane phenotype of precursor and amplifier cells. *J.exp.Med.* 145: 793 (1977).

- 134 Feldman, M.: Cell interactions in the immune response *in vitro*. II. The requirement of macrophages in lymphoid cell collaboration. *J.exp.Med.* 135: 1049 (1972).
- 135 Feldman, M.; Basten, A.: Cell interaction in the immune response *in vitro*. III. Specific collaboration across a cell impermeable membrane. *J.exp.Med.* 136: 49 (1972).
- 136 Feldmann, M.: Cell interactions in the immune response *in vitro*. V. Specific collaboration via complexes of antigen and thymus-derived immunoglobulin. *J.exp.Med.* 136: 737 (1972).
- 137 Feldmann, M.; Pepys, M.B.: Role of C3 in lymphocyte co-operation *in vitro*. *Nature, Lond.* 249: 159 (1973).
- 138 Fidler, J.M.; Chiscon, M.O.; Golub, E.S.: Functional development of the interacting cells in the immune response. II. Development of immunocompetence to heterologous erythrocytes *in vitro*. *J.Immun.* 109: 136 (1972).
- 139 Figueredo, M.A.; Palomino, P.; Ortiz, F.: Lymphocyte response to phytohaemagglutinin in the presence of serum from pregnant women. Correlation with serum levels of alpha-feto-protein. *Clin.exp.Immunol.* 37: 140 (1979).
- 140 Fisher, M.S.; Kripke, M.L.: Further studies on the tumor-specific suppressor cells induced by ultraviolet radiation. *J.Immun.* 121: 1139 (1978).
- 141 Folch, H.; Waksman, B.H.: Regulation of lymphocyte responses *in vitro*. V. Suppressor activity of adherent and non-adherent rat lymphoid cells. *Cell.Immunol.* 9: 12 (1973).
- 142 Folch, H.; Yoshinaga, M.; Waksman, B.H.: Regulation of lymphocyte responses *in vitro*. III. Inhibition by adherent cells of the T-lymphocyte response to phytohemagglutinin. *J.Immun.* 110: 835 (1973).
- 143 Forni, G.; Green, I.: Heterologous sera. A target for *in vitro* cell-mediated cytotoxicity. *J.Immun.* 116: 1561 (1976).
- 144 Forsgren, A.; Svedjelund, A.; Wigzell, H.: Lymphocyte stimulation by protein A of *Staphylococcus aureus*. *Eur.J.Immunol.* 6: 207 (1976).
- 145 Friedberg, S.H.; Weissman, I.L.: Lymphoid tissue architecture. II. Ontogeny of peripheral T and B cells in mice: Evidence against Peyer's Patches as the site of generation of B cells. *J.Immun.* 113: 1477 (1974).
- 146 Fuchs, T.; Hammarstrom, L.; Smith, E.; Brundin, J.: *In vivo* suppression of uterine lymphocytes during early human pregnancy. *Acta obstet. gynec. scand.* 56: 151 (1977).
- 147 Fujinami, R.S.; Paterson, P.Y.; Parmely, M.J.; Thompson, J.S.; Goeken, N.E.: Lack of suppressive effect of a-fetoprotein on development of experimental allergic encephalomyelitis in rats. *Nature, Lond.* 77: 3635 (1976).
- 148 Furth, R. van; Adinolfi, M.: *In vitro* synthesis of the foetal alpha₂ globulin in man. *Nature, Lond.* 222: 1296 (1969).
- 149 Gelfand, M.C.; Elfenbein, G.J.; Frank, M.M.; Paul, W.E.: Ontogeny of B lymphocytes. II. Relative rates of appearance of lymphocytes bearing surface immunoglobulin and complement receptors. *J.exp.Med.* 139: 1125 (1974).
- 150 Germain, R.N.; Ju, S.T.; Kipps, T.J.; Benacerraf, B.; Dorf, M.E.: Shared idiotypic determinants on antibodies and T cell derived suppressor factor specific for the random terpolymer L-glutamic acid⁹⁰-L-alanine³⁰-L-tyrosine¹⁰. *J.exp.Med.* 149: 613 (1979).
- 151 Gershon, R.; Kondo, K.: Cell interactions in the induction of tolerance: The role of thymic lymphocytes. *Immunology* 18: 723 (1970).

- 152 Gershon, R.K.: T cell control of antibody production. *Contemp. Top. Immunobiol.* 3: 1 (1974).
- 153 Gershon, R.K.; Horowitz, M.; Kemp, J.D.; Murphy, D.B.; Murphy, E.D.: The cellular site of immunoregulatory breakdown in the lpr mutant mouse; in Rose, Bigazzi, Warner, Genetic control of autoimmune disease, p.223 (Elsevier/North Holland, Amsterdam 1978).
- 154 Gershon, R.K.; Eardley, D.D.; Ptak, W.: Functional inactivation of suppressor T cells by heat-killed macrophages. *Nature, Lond.* 262: 216 (1976).
- 155 Gershon, R.K.; Kondo, K.: Cell interactions in the induction of tolerance: the role of thymic lymphocytes. *Immunology* 18: 273 (1970).
- 156 Gershon, R.K.; Kondo, K.: Infectious immunological tolerance. *Immunology* 21: 903 (1971).
- 157 Gershwin, M.E.; Castles, J.J.; Ahmad, A.; Makishima, R.: The influence of alpha-fetoprotein on Moloney sarcoma virus oncogenesis. Evidence for generation of antigen nonspecific suppressor T cells. *J. Immun.* 121: 2292 (1978).
- 158 Gidlund, M.; Orn, A.; Wigzell, H.; Senik, A.; Gresser: Enhanced NK cell activity in mice injected with interferon and interferon inducers. Part I. *Nature, Lond.* 273: 759 (1978).
- 159 Gidlund, M.; Ojo, E.E.; Orn, A.; Wigzell, H.; Murgita, R.A.: Severe suppression of the B-cell system has no impact on the maturation of natural killer cells in mice. *Scand. J. Immunol.* 9: 167 (1979).
- 160 Gitlin, D.: Normal biology of alpha-fetoprotein. *Ann. N.Y. Acad. Sci.* 259: 7 (1975).
- 161 Gitlin, D.; Perricelli, A.: Synthesis of serum albumin, prealbumin, alpha-fetoprotein, alpha-antitrypsin and transferrin by the human yolk sac. *Nature, Lond.* 228: 995 (1970).
- 162 Globerson, A.; Umiel, T.: Ontogeny of suppressor cells. II. Suppression of graft-versus-host and mixed leucocyte culture responses by embryonic cells. *Transplantation* 26: 438 (1978).
- 163 Globerson, A.; Zinkernagel, R.M.; Umiel, T.: Immunosuppression by embryonic liver cells. *Transplantation* 20: 480 (1975).
- 164 Goeken, N.E.; Thompson, J.S.: Conditions affecting the immunosuppressive properties of human alpha-fetoprotein. *J. Immun.* 119: 139 (1977).
- 165 Goidl, E.A.; Siskind, G.W.: Ontogeny of B-lymphocyte function. I. Restricted heterogeneity of the antibody response of B lymphocytes from neonatal and fetal mice. *J. exp. Med.* 140: 1285 (1974).
- 166 Goidl, E.A.; Klass, J.; Siskind, G.W.: Ontogeny of B lymphocyte function. II. Ability of endotoxin to increase the heterogeneity of affinity of the immune response of B lymphocytes from fetal mice. *J. exp. Med.* 143: 1503 (1976).
- 167 Goidl, E.A.; Wigzell, H.; Murgita, R.A.: Studies on the mechanisms of alpha-fetoprotein induction of immune suppressive activity; in Siskind, Litwin, Weksler, Developmental immunobiology. Proc. Fifth Irwin Strasburger Memorial Seminar on Immunology, p.35 (Grune & Stratton, New York 1979).
- 168 Goldstein, A.L.; Guha, A.; Howe, M.L.; White, A.: Ontogenesis of cell-mediated immunity in murine thymocytes and spleen cells and its acceleration by thymosin, a thymic hormone. *J. Immun.* 106: 773 (1971).
- 169 Golub, E.S.: Brain-associated theta antigen. Reactivity of rabbit anti-mouse brain with mouse lymphoid cells. *Cell. Immunol.* 2: 353 (1971).

- 170 Goodman, S.A.; Vitetta, E.S.; Uhr, J.W.: Cell surface immunoglobulin XIII. Distribution of IgM and IgD-like molecules on small and large cells of mouse spleen. *J. Immunol.* 114: 1646 (1975).
- 171 Goodwin, J.S.; Bankhurst, A.D.; Messner, R.P.: Suppression of human T-cell mitogenesis by prostaglandin. Existence of a prostaglandin-producing suppressor cell. *J. exp. Med.* 146: 1719 (1977).
- 172 Goodwin, J.S.; Webb, D.R.: Regulation of the immune response by prostaglandins. *Clin. Immunol. Immunopathol.* 15: 106 (1980).
- 173 Gorczynski, R.M.; Feldmann, M.: B cell heterogeneity-difference in the size of B lymphocytes responding to T-dependent and T-independent antigens. *Cell. Immunol.* 18: 88 (1975).
- 174 Greene, M.I.; Pierres, A.; Dorf, M.E.; Benacerraf, B.: The I-J subregion codes for determinants on suppressor factor(s) which limit the contact sensitivity response to picryl chloride. *J. exp. Med.* 146: 293 (1977).
- 175 Gupta, S.; Good, R.A.: Alpha-fetoprotein and human lymphocyte subpopulations. *J. Immunol.* 118: 405 (1977).
- 176 Gupta, S.; Goel, Z.; Grieco, M.H.: Fetuin. *In vitro* effect on sheep erythrocyte rosette formation with human T lymphocytes. *Int. Archs Allergy Appl. Immun.* 52: 273 (1976).
- 177 Gustine, D.L.; Zimmerman, E.F.: Developmental changes in microheterogeneity of fetal plasma glycoproteins. *Biochem. J.* 132: 541 (1973).
- 178 Gustine, D.L.; Zimmerman, E.F.: Developmental changes in microheterogeneity of foetal plasma glycoproteins in mice. *Biochem. J.* 132: 541 (1973).
- 179 Hamilton, M.S.; Hellstrom, I.: Altered immune responses in pregnant mice. *Transplantation* 23: 423 (1977).
- 180 Hammerling, U.: B lymphocyte differentiation and regulatory signals; in Siskind, Litwin, Weksler, *Developmental immunobiology. Proc. Fifth Irwin Strasburger Memorial Seminar on Immunology*, p. 59 (Grune & Stratton, New York 1979).
- 181 Hammerling, U.; Chin, A.F.; Abbott, J.: Ontogeny of B lymphocytes. Sequence of B-cell differentiation from surface-immunoglobulin negative precursors to plasma cells. *Proc. natn. Acad. Sci. USA* 73: 2008 (1976).
- 182 Hammerling, U.; Chin, A.F.; Abbott, J.; Scheid, M.P.: Ontogeny of murine B lymphocytes. I. Induction of phenotypic conversion of Ia⁻ to Ia⁺ lymphocytes. *J. Immunol.* 115: 1425 (1975).
- 183 Hand, T.L.; Ceglowski, W.S.; Damrongsak, D.; Friedman, H.: Development of antibody-forming cells in neonatal mice. Stimulation and inhibition by calf thymus fractions. *J. Immunol.* 105: 442 (1970).
- 184 Hansson, M.; Karre, K.; Kiessling, R.; Roder, J.C.; Andersson, B.; Hayry, P.: Natural NK cell targets in the mouse thymus. Characteristics of the sensitive cell population. *J. Immunol.* 123: 765 (1979).
- 185 Hansson, M.; Kiessling, R.; Anderson, B.; Karre, K.; Roder, J.C.: NK-cell sensitive T-cell subpopulation in thymus. Inverse correlation to host NK activity. *Nature, Lond.* 278: 174 (1979).
- 185a Hansson, M.; Kiessling, R.; Andersson, B.: Human fetal thymus and bone marrow contain target cells for natural killer cells. *Eur. J. Immunol.* 11: 8 (1981).
- 186 Hardy, B.; Mozes, E.; Danon, D.: Comparison of the immune response potential of

- newborn mice to T-dependent and T-independent synthetic polypeptides. *Immunology* 30: 261 (1976).
- 187 Hardy, B.; Globerson, A.; Danon, D.: Ontogenetic development of the reactivity of macrophages to antigenic stimulation. *Cell. Immunol.* 9: 282 (1973).
 - 188 Hardy, B.; Mozes, E.: Expression of T cell suppressor activity in the immune responses of newborn mice to a T-independent synthetic polypeptide. *Immunology* 35: 757 (1978).
 - 189 Harrison, M.R.: Maternal Immunocompetence. II. Proliferative responses of maternal lymphocytes *in vitro* and inhibition by serum from pregnant rats. *Scand.J. Immunol.* 5: 881 (1976).
 - 190 Hassoux, R.; Poupon, M.F.; Uriel, J.: Lack of inhibition by mouse alpha-feto-proteins (AFP) of *in vitro* induced lymphocyte blastogenesis. *Ann. Immunol., Paris* 129C: 275 (1978).
 - 191 Hellstrom, I.; Hellstrom, K.E.; Allison, A.C.: Neonatally induced allograft tolerance may be mediated by serum-borne factors. *Nature, Lond.* 230: 49 (1971).
 - 192 Hellstrom, K.E.; Hellstrom, I.; Braun, J.: Abrogation of cellular immunity to antigenically foreign mouse embryonic cells by a serum factor. *Nature, Lond.* 224: 914 (1969).
 - 193 Herberman, R.B.; Nunn, M.E.; Lavrin, D.H.: Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. *Int.J.Cancer* 16: 216 (1975).
 - 194 Herberman, R.B.; Nunn, M.E.; Holden, H.T.; Lavrin, D.H.: Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int.J.Cancer* 16: 230 (1975).
 - 195 Heron, I.: Prolonged survival of heart allografts transplanted in rats and rabbits following pregnancy. *Transplantation* 14: 551 (1972).
 - 196 Herzenberg, L.A.; Okumura, K.; Cantor, H.; Sato, V.L.; Shen, F.W.; Boyse, E.A.; Herzenberg, L.A.: T cell regulation of antibody responses. Demonstration of allotype-specific helper T cells and their specific removal by suppressor T cells. *J. exp. Med.* 144: 330 (1976).
 - 197 Hirai, Y.; Nisonoff, A.: Selective suppression of the major idiotypic component of an antihapten response by soluble T cell-derived factors with idiotypic or anti-idiotypic receptors. *J.exp. Med.* 151: 1213 (1980).
 - 198 Hirst, J.A.; Beverley, P.C.L.; Kisielow, P.; Hoffman, M.K.; Oettgen, H.E.: Markers of T cells function on mouse spleen cells. *J. Immun.* 115: 1555 (1975).
 - 199 Hooper, D.C.; Murgita, R.A.: Evidence for two distinct neonatal suppressor cell populations. *Fed. Proc.* 39: 354 (1980).
 - 200 Hooper, D.C.; Delovitch, T.L.; Wigzell, H.; Murgita, R.A.: Analysis of an immunoregulatory suppressor T cell system in newborn mice; in Kaplan, The molecular basis of immune cell function, p.676 (Elsevier/North-Holland Biomedical Press, Amsterdam 1979).
 - 201 Howe, C.: Lymphocyte physiology during pregnancy: *in vivo* and *in vitro* studies; in Edwards, Howe, Johnson, *Int. Conf. Immunobiology of Trophoblast*, p.131 (Cambridge University Press, Cambridge 1974).
 - 202 Howe, M.L.; Manziello, B.: Ontogenesis of the *in vitro* response of murine lymphoid cells to cellular antigens and phytomitogens. *J. Immun.* 109: 534 (1972).

- 203 Hsu, C.C.S.: Peripheral blood lymphocyte responses to phytohemagglutinin and pokeweed mitogen during pregnancy. *Proc.Soc.exp.Biol.Med.* 146: 771 (1974).
- 204 Hsu, C.C.S.; Waithe, W.I.; Hathaway, P.; Hirschhorn, K.: The effects of foetus on lymphocytes. Lymphocyte-stimulating property. *Clin. exp. Immunol.* 15: 427 (1973).
- 205 Huber, B.; Gershon, R.K.; Cantor, H.: Identification of a B-cell surface structure involved in antigen-dependent triggering. Absence of this structure on B cells from CBA/N mutant mice. *J.exp.Med.* 145: 10 (1977).
- 206 Irie, R.F.; Irie, K.; Morton, D.L.: Natural antibody in human serum to a neoantigen in human cultured cells grown in fetal bovine serum. *J.natn. Cancer Inst.* 4: 1051 (1974).
- 207 Isakov, N.; Segal, S.; Feldman, M.: Immune surveillance and feto-maternal relationships: suppression of immune functions by mouse amniotic fluid. *Proc. 12th Int. Leucocyte Culture Conf.*, 1978.
- 208 Jacobson, E.B.; Herzenberg, L.A.: Active suppression of immunoglobulin allotype synthesis. I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL \times BALB/c) F mice. *J.exp.Med.* 135: 1151 (1972).
- 209 Jacobson, E.B.; Herzenberg, L.A.; Riblet, R.; Herzenberg, L.A.: Active suppression of immunoglobulin allotype synthesis. II. Transfer of suppressing factor with spleen cells. *J.exp. Med.* 135: 1163 (1972).
- 210 Jalanko, H.; Engvall, E.; Ruoslahti, E.: Immunochemical properties of alpha-fetoprotein (AFP) and antibodies to autologous AFP. *Immunol. Commun.* 7: 209 (1978).
- 211 Janeway, C.A., Jr.; Murgita, R.A.; Weinbaum, F.I.; Asofsky, R.; Wigzell, H.: Evidence for an immunoglobulin-dependent antigen-specific helper T cell. *Proc. natn.Acad.Sci.USA* 74: 4582 (1977).
- 212 Janeway, C.A.; Sharrow, S.O.; Simpson, E.: T cell populations with different functions. *Nature, Lond.* 253: 544 (1975).
- 213 Jayawardena, A.N.; Waksman, B.H.: Suppressor cells in experimental trypanosomiasis. *Nature, Lond.* 265: 539 (1977).
- 214 Jennings, J.J.; Rittenberg, M.B.: Evidence for separate subpopulations of B cells responding to T-independent and T-dependent antigens. *J.Immun.* 117: 1749 (1976).
- 215 Kagnoff, M.F.; Billings, P.; Cohen, M.: Functional characteristics of Peyer's patch lymphoid cells. II. Lipopolysaccharide is thymus dependent. *J.exp. Med.* 120: 305 (1974).
- 216 Kamarck, M.E.; Göttlieb, P.D.: Expression of thymocyte surface alloantigens in the fetal mouse thymus *in vivo* and in organ culture. *J.Immun.* 119: 407 (1977).
- 217 Kamo, I.; Friedman, H.: Immunosuppression and the role of suppressive factors in cancer. *Adv. Cancer Res.* 25: 271 (1977).
- 218 Kapp, J.A.; Pierce, C.W.; De la Croix, F.; Benacerraf, B.: Immunosuppressive factor(s) extracted from lymphoid cells of nonresponder mice primed with L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT). I. Activity and antigenic specificity. *J.Immun.* 116: 305 (1976).
- 219 Kasakura, S.: A factor in maternal plasma during pregnancy that suppresses the reactivity of mixed leukocyte cultures. *J.Immun.* 107: 1296 (1971).
- 220 Kasakura, S.: Is cortisol responsible for inhibition of MLC reactions by pregnancy plasma? *Nature, Lond.* 246: 496 (1973).

- 221 Katz, S.K.; Parker, D.; Turk, J.L.: B-cell suppression of delayed hypersensitivity reactions. *Nature, Lond.* 251: 550 (1974).
- 222 Kearney, J.F.; Cooper, M.D.; Klein, J.; Abney, E.R.; Parkhouse, R.M.E.; Lawton, A.R.: Ontogeny of Ia and IgD on IgM-bearing B lymphocytes in mice. *J.exp.Med.* 146: 297 (1977).
- 223 Kedar, E.; Schwartzbach, M.; Unger, E.; Lupu, T.: Characteristics of suppressor cells induced by fetal bovine serum in murine lymphoid cell cultures. *Transplantation* 26: 63 (1978).
- 224 Kedar, E.; Schwartzbach, M.: Further characterization of suppressor lymphocytes induced by fetal calf serum in murine lymphoid cell cultures. Comparison with *in vitro*-generated cytotoxic lymphocytes. *Cell. Immunol.* 43: 326 (1979).
- 225 Keller, R.: Major changes in lymphocyte proliferation evoked by activated macrophages. *Cell. Immunol.* 17: 542 (1975).
- 226 Keller, R.H.; Calvanico, N.J.; Tomasi, T.B.: Immunosuppressive properties of AFP: Role of estrogens; in Fishman, Sell, *Onco-Developmental gene expression*, p.287 (Academic Press, New York 1976).
- 227 Kerbel, R.S.; Eidinger, D.: Enhanced immune responsiveness to a thymus-independent antigen early after adult thymectomy. Evidence for short-lived inhibitory thymus-derived cells. *Eur.J.Immunol.* 2: 114 (1972).
- 228 Kerbel, R.S.; Blakeslee, D.: Rapid adsorption of a foetal calf serum component by mammalian cells in culture. A potential source of artifacts in studies of antisera to cell-specific antigens. *Immunology* 31: 881 (1976).
- 229 Kiessling, R.; Wigzell, H.: An analysis of the murine NK cell as to structure, function, and biological relevance. *Immunol. Rev.* 44: 165 (1979).
- 230 Kiessling, R.; Klein, E.; Wigzell, H.: Natural killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur.J.Immunol.* 5: 112 (1975).
- 231 Kiessling, R.; Klein, E.; Pross, H.; Wigzell, H.: Natural killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *Eur.J.Immunol.* 5: 117 (1975).
- 232 Kirchner, H.; Fernbach, B.R.; Herberman, R.B.: Macrophages suppressing T and B mitogen responses and the mixed leucocyte reaction; in Oppenheim, Rosenstreuch, *Mitogens in immunobiology*, p.495 (Academic Press, New York 1975).
- 233 Kithier, K.; Poulik, M.D.: Comparative studies of bovine alpha-fetoprotein and fetuin. *Biochim. biophys. Acta* 278: 505 (1972).
- 234 Klinman, N.R.; Press, J.L.: The characterization of the B-cell repertoire specific for 24-dinitrophenyl and 2, 4, 6-trinitrophenyl determinants in neonatal Balb/c mice. *J.exp.Med.* 141: 1133 (1978).
- 235 Knight, S.C.; Thorbecke, G.J.: Ontogeny of cellular immunity: Development in rat thymocytes of mixed lymphocyte reactivity to allogeneic and xenogeneic cells. *Cell. Immunol.* 2: 91 (1971).
- 236 Knospe, W.H.; Ward, P.H.; Dors, R.; Fried, W.; Sasseti, R.J.; Trobaugh, F.E.: Fetuin stimulation of hematopoietic stem cells (HSC). *Blood* 38: 818 (1971).
- 237 Kolsch, E.; Mengersen, R.; Weber, G.: Suppressor T cells in low zone tolerance; in Sercarz, Williamson, Fox, *The immune system. Genes, receptors, signals*, p.447 (Academic Press, New York 1974).

- 238 Kontiainen, S.; Feldman, M.: Suppressor cell induction *in vitro*. I. Kinetics of induction of antigen specific suppressor cells. *Eur.J.Immunol.* 6: 296 (1976).
- 239 Kontiainen, S.; Feldman, M.: Suppressor cell induction *in vitro*. III. Antigen-specific suppression by supernatants of suppressor cells. *Eur.J.Immunol.* 7: 310 (1977).
- 240 Kurland, J.I.; Kincade, P.W.; Moore, M.A.S.: Regulation of B lymphocyte clonal proliferation by stimulatory and inhibitory macrophage-derived factors. *J.exp.Med.* 146: 1420 (1977).
- 241 Labib, R.S.; Tomasi, T.B.: Immunosuppressive factors in mouse amniotic fluid and neonate serum. *Immunol.Commun.* 7: 323 (1978).
- 242 Landahl, C.A.: Ontogeny of adherent cells. I. Distribution and ontogeny of A cells particularly in the response to sheep erythrocytes *in vitro*. *Eur.J.Immunol.* 6: 130 (1976).
- 243 Lawler, S.D.; Reaves, B.R.; Ukaejiofo, E.O.: Interaction of maternal and neonatal cells in mixed lymphocyte cultures. *Lancet ii*: 1185 (1975).
- 244 Lawton, A.R.; Cooper, M.D.: Two new stages of antigen-independent B cell development in mice and humans; in Cooper, Dayton, Development of host defences, p.43 (Raven Press, New York 1977).
- 245 Lawton, A.R.; Asofsky, R.; Hylton, M.B.; Cooper, M.D.: Suppression of immunoglobulin class synthesis in mice. I. Effects of treatment with antibody to μ -chain. *J.exp.Med.* 135: 277 (1972).
- 246 Leberman, D.A.; Calkins, C.E.: Demonstration of active suppressor cells in spleens of young NZB mice. *Cell.Immunol.* 51: 419 (1980).
- 247 Lee, K.C.; Shiozawa, C.; Shaw, A.; Diener, E.: Requirement for accessory cells in the antibody response to T cell independent antigen *in vitro*. *Eur.J.Immunol.* 6: 63 (1976).
- 248 Leikin, S.: Depressed maternal lymphocyte response to phytohaemagglutinin in pregnancy. *Lancet i*: 43 (1972).
- 249 Lester, E.P.; Miller, J.B.; Yachnin, S.: Human alpha-fetoprotein as a modulator of human lymphocyte transformation. Correlation of biological potency with electrophoretic variants. *Proc.natn.Acad.Sci.USA* 73: 4645 (1976).
- 250 Lester, E.P.; Miller, J.B.; Yachnin, S.: Human alpha-fetoprotein. Immunosuppressive activity and microheterogeneity. *Immunol.Commun.* 7: 137 (1978).
- 251 Lester, E.P.; Miller, J.B.; Yachnin, S.: Human alpha-fetoprotein as a modulator of human lymphocyte transformation. Correlation of biological potency with electrophoretic variants. *Proc.natn.Acad.Sci.USA* 73: 4645 (1976).
- 252 Lester, E.P.; Miller, J.B.; Yachnin, S.: A postsynthetic modification of human alpha-fetoprotein controls its immunosuppressive potency. *Proc.natn.Acad.Sci.USA* 74: 3988 (1977).
- 253 Lester, E.P.; Miller, J.B.; Yachnin, S.: A postsynthetic modification of human alpha-fetoprotein controls its immunosuppressive potency. *Proc.natn.Acad.Sci.USA* 74: 3988 (1977).
- 254 Levine, M.A.; Claman, H.N.: Bone marrow and spleen. Dissociation of immunologic properties by cortisone. *Science* 167: 1515 (1970).
- 255 Levis, W.R.; Robbins, J.H.: Effect of glass-adherent cells on the blastogenic response of 'purified' lymphocytes to phytohemagglutinin. *Expl.Cell Res.* 61: 153 (1970).

- 256 Lewis, G.K.; Goodman, J.W.: Carrier-directed anti-hapten responses by B cell subsets. *J.exp.Med.* 146: 1 (1977).
- 257 Lewis, G.K.; Ranken, R.; Nitecki, D.E.; Goodman, J.W.: Murine B cell subpopulations responsive to T-dependent and T-independent antigens. *J.exp.Med.* 144: 382 (1976).
- 258 Lindahl-Kiessling, K.; Mattsson, A.; Skoog, V.: A factor in amniotic fluid inhibiting phytohemagglutinin (PHA) induced lymphocyte growth. *Life Sci.* 9: 1427 (1970).
- 259 Littman, B.H.; Alpert, E.; Rocklin, R.: The effect of purified alpha-fetoprotein on *in vitro* assays of cell-mediated immunity. *Cell.Immunol.* 30: 35 (1977).
- 260 Lu, C.Y.; Calamai, E.G.; Unanue, E.R.: A defect in the antigen-presenting function of macrophages from neonatal mice. *Nature, Lond.* 282: 327 (1979).
- 261 Luckenbach, G.A.; Kennedy, M.M.; Kelly, A.; Mandel, T.E.: Suppression of an *in vitro* humoral immune response by cultured fetal thymus cells. *Eur.J.Immunol.* 8: 8 (1978).
- 262 Luzzati, A.L.; Jacobson, E.B.: Serum immunoglobulin levels in nude mice. *Eur.J.Immunol.* 2: 473 (1972).
- 263 Manning, D.D.: Complete humoral immunosuppression of mice by rabbit anti-antibodies passing the murine placenta. *J.Immun.* 118: 1109 (1977).
- 264 Manning, D.D.; Jutila, J.W.: Immunosuppression of mice injected with heterologous anti-immunoglobulin heavy chain antisera. *J.exp.Med.* 135: 1316 (1972).
- 265 Maraz, A.; Petri, I.B.: Intrinsic alteration of lymphocyte reactivity in women with normal pregnancy or tumour of placental origin. *Cell.Immunol.* 10: 496 (1974).
- 266 Marbrook, J.: Primary immune responses in cultures of spleen cells. *Lancet ii*: 1279 (1967).
- 267 Martineau, R.S.; Johnson, J.S.: Normal mouse serum immunosuppressive activity: Action on adherent cells. *J.Immun.* 120: 1550 (1978).
- 268 Masseyeff, R.; Gilli, J.; Krebs, B.; Calluand, A.; Bonet, C.: Evolution of alpha-fetoprotein serum levels throughout life in humans and rats, and during pregnancy in rats. *Ann.N.Y. Acad.Sci.* 275: 17 (1975).
- 269 Mathieson, B.J.; Sharrow, S.O.; Campbell, P.S.; Asofsky, R.: An Lyt differentiated thymocyte subpopulation detected by flow microfluorometry. *Nature, Lond.* 277: 478 (1979).
- 270 McCullagh, P.: Role of the thymus in suppression of the immune responses in newborn rats. *Aust.J.exp.Biol.med.Sci.* 52: 413 (1975).
- 271 McCullagh, P.: Radiosensitivity of suppressor cells in newborn rats. *Aust.J.exp.Biol.med.Sci.* 53: 399 (1975).
- 272 McCullagh, P.: Transplacental influence of thymus. *J.exp.Med.* 145: 772 (1977).
- 273 McDougal, J.S.; Shen, F.W.; Elster, P.: Generation of T helper cells *in vitro*. V. Antigen-specific Lyt⁺ T cells mediate the helper effect and induce feedback suppression. *J.Immun.* 122: 437 (1979).
- 274 McIntyre, J.A.; Faulk, W.P.: Antigens of human trophoblast. Effects of heterologous antitrophoblast sera on lymphocyte responses *in vitro*. *J.exp.Med.* 149: 824 (1979).
- 275 McIntyre, J.A.; Faulk, W.P.: Suppression of mixed lymphocyte cultures by antibodies against human trophoblast membrane antigens. *Transplant. Proc.* 10: 919 (1978).
- 276 McIntyre, J.A.; Faulk, W.P.: Trophoblast modulation of maternal allogeneic recognition. *Proc.natn.Acad.Sci. USA* 76: 4029 (1979).

- 277 McKearn, J.P.; Quintano, J.: Ontogeny of murine B-cell responses to thymus-independent trinitrophenyl antigens. *Cell Immunol.* 44: 367 (1979).
- 278 McKenzie, I.F.C.; Potter, T.: Murine lymphocyte surface antigens. *Adv. Immunol.* 27: 179 (1979).
- 279 Medawar, P.B.; Sparrow, E.M.: The effects of adrenocortical hormones, adrenocorticotrophic hormone and pregnancy on skin transplantation immunity in mice. *J. Endocr.* 14: 240 (1956).
- 280 Melchers, F.; Boehmer, H. von; Phillips, R.A.: B-lymphocyte subpopulations in the mouse. Organ distribution and ontogeny of immunoglobulin-synthesizing and mitogen-sensitive cells. *Transplant. Rev.* 25: 26 (1975).
- 281 Melchers, F.; Andersson, J.; Phillips, R.A.: Ontogeny of murine B lymphocytes: Development of Ig synthesis and of reactivities to mitogens and to anti-Ig antibodies. *Cold Spring Harbor Symp. Quant. Biol.* 16: 147 (1977).
- 282 Mendelsohn, J.; Skinner, A.; Kornfeld, S.: The rapid induction by phytohemagglutinin of increased-aminoisobutyric acid uptake by lymphocytes. *J. clin. Invest.* 50: 818 (1971).
- 283 Merritt, K.; Galton, M.: Antibody formation during pregnancy. *Transplantation* 7: 562 (1969).
- 284 Minato, N.; Reid, L.; Cantor, H.; Lengyel, P.; Bloom, B.R.: Mode of regulation of natural killer cell activity by interferon. *J. exp. Med.* 152: 124 (1980).
- 285 Mizejewski, G.J.; Allen, R.P.: Immunotherapeutic suppression in transplantable solid tumors. *Nature, Lond.* 250: 50 (1974).
- 286 Mizewski, G.J.; Grimley, P.M.: Aburtogenic activity of antiserum to alpha-foeto-protein. *Nature, Lond.* 259: 222 (1976).
- 287 Moller, G.: The role of macrophages in the immune response. *Immunol. Rev.* 40: (1978).
- 288 Moller, G.: Suppressor T lymphocytes. *Transplant. Rev.* 26: 1 (1975).
- 289 Morton, H.; Hegh, V.; Clunie, G.J.A.: Immunosuppression detected in pregnant mice by rosette inhibition test. *Nature, Lond.* 249: 459 (1974).
- 290 Moore, M.A.S.; Owen, J.J.T.: Experimental studies on the development of the thymus. *J. exp. Med.* 126: 715 (1967).
- 291 Moore, M.A.S.; Metcalf, D.: Ontogeny of the haemopoietic system. Yolk sac origin in *in vivo* and *in vitro* clony-forming cells in the developing embryo macrophage. *Br. J. Haemat.* 18: 279 (1970).
- 292 Moretta, L.; Ferrarini, M.; Mingari, M.C.; Moretta, A.; Webb, S.R.: Subpopulations of human T cells identified by receptors for immunoglobulins and mitogen responsiveness. *J. Immun.* 117: 2171 (1976).
- 293 Moretta, L.; Webb, S.R.; Grossi, C.E.; Lydyard, P.M.; Cooper, M.D.: Functional analysis of two human T cell sub-populations. Help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. *J. exp. Med.* 146: 184 (1977).
- 294 Morse, H.C.; Prescott, B.; Cross, S.S.; Stashak, P.W.; Baker, P.J.: Regulation of the antibody response to type III pneumococcal polysaccharide. V. Ontogeny of factors influencing the magnitude of the plaque-forming cell response. *J. Immun.* 116: 279 (1976).
- 295 Morse, J.H.; Witte, L.D.; Goodman, D.S.: Inhibition of lymphocyte proliferation stimulated by lectins and allogeneic cells by normal plasma lipoproteins. *J. exp. Med.* 146: 1791 (1977).

- 296 Morse, J.H.; Stearns, G.; Arden, J.; Agosto, G.M.; Canfield, R.E.: The effects of crude and purified human gonadotropin on *in vitro* stimulated human lymphocyte cultures. *Cell.Immunol.* 25: 178 (1976).
- 297 Mortensen, R.F.: C-reactive (CRP)-mediated inhibition of the induction of *in vitro* antibody formation. I. T cell dependent of the inhibition. *Cell.Immunol.* 44: 270 (1979).
- 298 Mortensen, R.F.; Osmand, A.P.; Gewurz, H.: Effects of C-reactive protein on the lymphoid system. I. Binding to thymus-dependent lymphocytes and alteration of their functions. *J.exp.Med.* 141: 821 (1975).
- 299 Mortensen, R.F.; Braun, D.; Gewurz, H.: Effects of C-reactive protein on lymphocyte functions. III. Inhibition of antigen-induced lymphocyte stimulation and lymphokine production. *Cell.Immunol.* 28: 59 (1977).
- 300 Mosier, D.E.: Ontogeny of mouse lymphocyte function. I. Paradoxical elevation of reactivity to allogeneic cells and phytohemagglutinin in Balb/c fetal thymocytes. *J.Immun.* 112: 305 (1974).
- 301 Mosier, D.E.; Cohen, P.L.: Ontogeny of mouse T-lymphocyte function. *Fed.Proc.* 34: 137 (1975).
- 302 Mosier, D.E.: Ontogeny of T cell function in the neonatal mouse; in Cooper, Dayton, Development of host defences, p.115 (Raven Press, New York 1977).
- 303 Mosier, D.E.; Coppleson, L.W.: A three-cell interaction required for the induction of the primary immune response *in vitro*. *Proc.natn.Acad.Sci. USA* 61: 542 (1968).
- 304 Mosier, D.E.; Tigelaar, R.E.; Cohen, P.L.: Ontogeny of *in vitro* correlates of graft-versus-host reactions. *Transplant. Proc.* 8: 371 (1976).
- 305 Mosier, D.E.; Johnson, B.M.: Ontogeny of mouse lymphocyte function. II. Development of the ability to produce antibody is modulated by T lymphocytes. *J.exp.Med.* 141: 216 (1975).
- 306 Mosier, D.E.; Zitron, I.M.; Mond, J.J.; Ahmed, A.; Scher, I.; Paul, W.E.: Surface immunoglobulin D as a functional receptor for a subclass of B lymphocytes. *Immunol. Rev.* 37: 89 (1977).
- 307 Mosier, D.E.; Zitron, I.M.; Mond, J.J.; Paul, W.E.: Requirements for the induction of antibody formation in the newborn mouse; in Siskind, Litwin, Weksler, Developmental immunobiology. *Proc. Fifth Irwin Strasburger Memorial Seminar on Immunology*, p.25 (Grune & Stratton, New York 1979).
- 308 Mosier, D.E.; Mathieson, B.J.; Campbell, P.S.: Ly phenotype and mechanism of action of mouse neonatal suppressor T cells. *J.exp.Med.* 146: 59 (1977).
- 309 Muchmore, A.V.; Blaese, R.M.: Immunoregulatory properties of fractions from human pregnancy urine. Evidence that human chronic gonadotropin is not responsible. *J.Immun.* 118: 881 (1977).
- 310 Mulder, A.M.; Durdik, J.M.; Toth, P.; Golub, E.S.: Leukemia in AKR mice. III. Size distribution of suppressor T cells in AKR leukemia and neonatal mice. *Cell. Immunol.* 40: 326 (1978).
- 311 Murgita, R.A.; Wigzell, H.: Selective immunoregulatory properties of alpha-feto-protein. *Ric.clin. Lab.* 9: 327 (1979).
- 312 Murgita, R.A.; Anderson, L.C.; Ruoslahti, E.; Kimura, A.; Wigzell, H.: Effects of human and mouse alpha-fetoprotein on immune responses. *Scand.J.Immunol.* 6: 731 (1976).

- 313 Murgita, R.A.: The immunosuppressive role of alpha-fetoprotein during pregnancy. *Scand.J.Immunol.* 5: 1003 (1976).
- 314 Murgita, R.A.; Wigzell, H.: The effects of mouse alpha-fetoprotein on T cell-dependent and T cell-independent immune responses *in vitro*. *Scand.J.Immunol.* 5: 1215 (1976).
- 315 Murgita, R.A.; Tomasi, T.B.: Suppression of the immune response by alpha-fetoprotein. I. The effect of mouse alpha-fetoprotein on the primary and secondary antibody response. *J.exp.Med.* 141: 269 (1975).
- 316 Murgita, R.A.; Tomasi, T.B.: Suppression of the immune response by alpha-fetoprotein. II. The effects of mouse alpha-fetoprotein on mixed lymphocyte reactivity and mitogen-induced lymphocyte transformation. *J.exp.Med.* 114: 440 (1975).
- 317 Murgita, R.A.; Mattioli, C.A.; Tomasi, T.B.: Production of a runting syndrome and selective A deficiency in mice by the administration of anti-heavy chain antisera. *J.exp.Med.* 138: 209 (1973).
- 318 Murgita, R.A.; Goidl, E.A.; Kontiainen, S.; Beverly, P.C.L.; Wigzell, H.: Adult T murine T cells activated *in vitro* by alpha-fetoprotein and naturally occurring I cells in newborn mice. Identity in function and cell surface differentiation antigens. *Proc.natn.Acad.Sci. USA* 75: 2897 (1978).
- 319 Murgita, R.A.; Hooper, D.C.; Delovitch, T.L.; Wigzell, H.: Newborn and adult alpha-fetoprotein-activated inhibitory T lymphocytes express identical all surface Ia determinants (in preparation).
- 320 Murgita, R.A.; Peck, A.B.; Wigzell, H.: Selective immunoregulatory properties of alpha-fetoprotein. *Fed.Proc.* 37: 1594 (1978).
- 321 Murgita, R.A.; Andersson, L.C.; Sherman, M.S.; Bennich, H.; Wigzell, H.: Effects of human alpha-fetoprotein on human B and T lymphocyte proliferation *in vitro*. *Clin.exp.Immunol.* 33: 347 (1978).
- 322 Murgita, R.A.; Goidl, E.A.; Kontiainen, S.; Wigzell, H.: Alpha-fetoprotein induces suppressor T cells *in vitro*. *Nature, Lond.* 267: 257 (1977).
- 323 Murphy, D.B.; Herzenberg, L.A.; Okumura, K.; McDevitt, H.O.: A new I subregion (I-J) marked by a locus (Ia-4) controlling surface determinants on suppressor T lymphocytes. *J.exp.Med.* 144: 699 (1976).
- 324 Murphy, D.B.; Herzenberg, L.A.; Okumura, K.; Herzenberg, L.A.; McDevitt, H.O.: A new I subregion (I-J) marked by a locus (Ia-4) controlling surface determinants on suppressor T lymphocytes. *J.exp.Med.* 144: 699 (1976).
- 325 Nabarra, B.; Papiernick, M.: Thymic subpopulations in young and adult mice. III. Qualitative and quantitative electron microscopic study of steroid resistant and steroid sensitive populations. *Cell.Immunol.* 51: 72 (1980).
- 326 Nadler, L.M.; Hodes, R.J.: Regulatory mechanisms in cell-mediated immune responses. II. Comparison of culture-induced and alloantigen-induced suppressor cells in MLR and CML. *J.Immun.* 118: 1886 (1977).
- 327 Nelson, D.S.; Gatti, R.A.: Humoral factors influencing lymphocyte transformations. *Prog.Allergy*, vol. 21, p.261 (Karger, Basel 1976).
- 328 Nelson, D.S.; Schneider, C.N.: Effect of normal mouse serum on mouse lymphocyte transformation. *Eur.J.Immunol.* 4: 79 (1974).
- 329 Newburger, P.E.; Hamaska, T.; Katz, D.H.: Potentiation of helper T cell function in IgE antibody responses by bacterial lipopolysaccharide (LPS). *J.Immun.* 113: 824 (1974).

- 330 Niederhuber, J.E.; Allen, P.: Role of I-region gene products in macrophage induction of an antibody response. II. Restriction at the level of T cell in recognition of I-J subregion macrophage determinants. *J.exp. Med.* 151: 1103 (1980).
- 331 Niederhuber, J.E.; Allen, P.; Mayo, L.: The expression of Ia antigenic determinants on macrophages required for the in vitro antibody. *J.Immun.* 122: 1342 (1979).
- 332 Noonan, F.P.; Halliday, W.J.; Morton, H.; Clunie, G.J.A.: Early pregnancy factor is immunosuppressive. *Nature, Lond.* 278: 649 (1979).
- 333 Norcross, M.A.; Smith, R.T.: Regulation of B cell proliferative responses to lipopolysaccharide by a subclass of thymus T cells. *J.exp.Med.* 145: 1299 (1977).
- 334 Nossal, G.H.V.; Pike, B.L.: Studies on the differentiation of B lymphocytes in the mouse. *Immunology* 25: 33 (1973).
- 335 Nunez, E.; Vallette, G.; Benassayag, C.; Jayle, M.: Comparative study on the binding of estrogens by human and rat serum proteins in development. *Biochem. biophys. Res. Commun.* 57: 126 (1974).
- 336 Nussenzweig, M.C.; Steinman, R.M.: Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reactions. *J.exp.Med.* 151: 1196 (1980).
- 337 Ogra, S.S.; Murgita, R.A.; Tomasi, T.B.: Immunosuppressive activity of mouse amniotic fluid. *Immunol. Commun.* 3: 497 (1974).
- 338 Okumura, K.; Takemori, T.; Tokuhisa, T.; Tada, T.: Specific enrichment of the suppressor T cell bearing I-J determinants. Parallel functional and serological characterizations. *J.exp.Med.* 146: 1234 (1977).
- 339 Okumura, K.; Herzenberg, L.A.; Murphy, D.B.; McDevitt, H.O.; Herzenberg, L.A.: Selective expression of H-2 (I-region) loci controlling determinants on helper and suppressor T lymphocytes. *J.exp. Med.* 144: 685 (1976).
- 340 Old, L.J.; Boyse, E.A.; Stockert, E.: Antigenic properties of experimental leukemias. I. Serological studies *in vitro* with spontaneous and radiation-induced leukemias. *J.natn. Cancer Inst.* 31: 977 (1963).
- 341 Olding, L.B.; Oldstone, M.B.A.: Lymphocytes from human newborns abrogate mitosis of their mother's lymphocytes. *Nature, Lond.* 249: 161 (1974).
- 342 Olding, L.B.; Oldstone, M.B.S.: Thymus-derived peripheral lymphocytes from human newborns inhibit division of their mother's lymphocytes. *J.Immun.* 116: 682 (1976).
- 343 Olding, L.B.; Benirschke, K.; Oldstone, M.B.A.: Inhibition of mitosis of lymphocytes from human adults by lymphocytes from human newborns. *Clin.Immunol. Immunopathol.* 3: 79.
- 344 Olding, L.B.; Murgita, R.A.; Wigzell, H.: Mitogen-stimulated lymphoid cells from human newborns suppress the proliferation of maternal lymphocytes across a cell-impermeable membrane. *J.Immun.* 119: 1109 (1977).
- 345 Oldstone, M.B.A.; Tishon, A.; Moretta, L.: Active thymus derived suppressor lymphocytes in human cord blood. *Nature, Lond.* 269: 333 (1977).
- 346 Olinesev, A.; Laky, M.; Popescu, D.E.; Dumitrescu, A.; Ganea, D.: The effect of alpha-fetoprotein on the immune response. *Scand.J.Immunol.* 8: 397 (1978).
- 347 Olsson, M.; Lundahl, G.; Ruoslahti, E.: Genetic control of alpha-fetoprotein synthesis in the mouse. *J.exp.Med.* 145: 819 (1977).
- 348 Opitz, H.-G.; Opitz, U.; Lenke, H.; Hewlett, G.; Schreml, W.; Flad, H.D.: The role of fetal calf serum in the primary immune response in vitro. *J.exp.Med.* 145: 1029 (1977).

- 349 Orn, A.; Gidlund, M.; Ojo, E.; Gronvik, K.-O.; Andersson, J.; Wigzell, H.; Murgita, R.A.; Senik, A.; Gressor, I.: Factors controlling the augmentation of natural killer cells; in Herberman. Natural cell-mediated immunity against tumours (Academic Press, New York, in press, 1980).
- 350 Orn, A.; Gidlund, M.; Wigzell, H.; Murgita, R.A.: AFP can block the enhancement of natural killer cell activity mediated by interferon inducers; In: Mechanism of Natural Immunity. Abstr. Fourth Int. Congr. Immunity, Paris 1980, p.11.6.15.
- 350a Orn, A.; Gidlund, M.; Wigzell, H.; Murgita, R.A.: Evidence that α -fetoprotein (AFP) suppresses natural killer (NK) cell activity in newborn mice (submitted for publication).
- 351 Owen, J.J.T.; Raff, M.C.: Studies on the differentiations of thymus-derived lymphocytes. *J.exp.Med.* 132: 1216 (1970).
- 352 Owen, J.J.T.; Raff, M.C.; Cooper, M.D.: Studies on the generation of B lymphocytes in the mouse embryo. *Eur.J.Immunol.* 5: 468 (1975).
- 353 Owen, J.J.T.; Cooper, M.D.; Raff, M.C.: *In vitro* generation of B lymphocytes in mouse foetal liver, a mammalian 'bursa equivalent'. *Nature, Lond.* 249: 361 (1974).
- 354 Parish, C.R.: Appearance of non-specific suppressor T cells during *in vitro* culture. *Immunology* 33: 397 (1977).
- 355 Parmely, M.J.; Thompson, J.S.: Effect of alpha-fetoprotein and other serum factors derived from hepatoma-bearing rats on the mixed lymphocyte response. *J.Immun.* 117: 1832.
- 356 Pavia, C.S.; Stites, D.P.: Immunosuppressive activity of murine newborn spleen cells. I. Selective inhibition of *in vivo* lymphocytes activation. *Cell.Immunol.* 42: 48 (1979).
- 357 Pavia, E.S.; Stites, D.P.: Humoral and cellular regulation of alloimmunity in pregnancy. *J.Immun.* 123: 2194 (1979).
- 358 Peck, A.B.; Andersson, L.C.; Wigzell, H.: Secondary *in vitro* responses of T lymphocytes to non-H-2 alloantigen. Self-H-2 restricted responses induced in heterologous serum are not dependent on primary-stimulating non-H-2 alloantigens. *J.exp.Med.* 145: 802 (1977).
- 359 Peck, A.B.; Murgita, R.A.; Wigzell, H.: Cellular and genetic restrictions in the immunoregulatory activity of alpha-fetoprotein. I. Selective inhibition of anti-Ja associated proliferative reactions. *J.exp.Med.* 147: 667 (1978).
- 360 Peck, A.B.; Janeway, C.A., Jr.; Wigzell, H.; Andersson, L.C.: Environmental and genetic control of T cell activation. *Transplant.Rev.* 35: 146 (1977).
- 361 Peck, A.B.; Murgita, R.A.; Wigzell, H.: Cellular and genetic restrictions in the immunoregulatory activity of alpha-fetoprotein. II. Alpha-fetoprotein-induced suppression of cytotoxic T lymphocyte development. *J.exp.Med.* 147: 360 (1978).
- 362 Pepys, M.M.: Role of complement in induction of the allergic response. *Nature new Biol.* 237: 157 (1972).
- 363 Pepys, M.B.: Role of complement in induction of antibody production *in vivo*. Effect of cobra factor and other C3-reactive agents on thymus-dependent and thymus-independent antibody responses. *J.exp.Med.* 140: 126 (1974).
- 364 Persellin, R.; Thoi, L.: Human polymorphonuclear leukocyte phagocytosis in pregnancy. Development of inhibitory during gestation and recovery in the postpartum period. *Am.J.Obstet.Gynec.* 134: 250 (1979).
- 365 Phillips, R.A.; Melchers, F.: Appearance of functional lymphocytes in fetal liver. *J.Immun.* 117: 1099 (1976).

- 366 Pickel, K.; Hoffman, M.K.: The Ly phenotype of suppressor T cells arising in mice subjected to a graft-versus-host reaction. *J.exp. Med.* 145: 1169 (1977).
- 367 Pilarski, L.M.: Ontogeny of cell-mediated immunity. I. Early development of allo-antigen-specific cytotoxic T-cell precursors in postnatal mice. *J.exp. Med.* 146: 887 (1977).
- 368 Pilarski, L.M.: A requirement for antigen-specific helper cells in the generation of cytotoxic T cells from thymocyte precursors. *J.exp. Med.* 145: 709 (1977).
- 369 Playfair, J.H.L.: Strain differences in the immune response of mice. I. The neonatal response to sheep red blood cells. *Immunology* 15: 35 (1978).
- 370 Playfair, J.H.L.; Purves, E.C.: Separate thymus dependent and thymus independent antibody forming cell precursors. *Nature new Biol.* 231: 149 (1971).
- 371 Polet, H.; Spieker-Polet, H.: Mechanism of the growth-promoting effect of serum albumin on concanavalin A-activated lymphocytes: Protective effect of the plasma proteins. *J.Immun.* 117: 1275 (1976).
- 372 Pope, B.L.; Whitney, R.B.; Levy, J.G.: Two distinct populations of suppressor cells in the spleens of mice bearing methylcholanthrene-induced tumors. *J.Immun.* 120: 2033 (1978).
- 373 Powell, A.E.: Maternal lymphocytes. Suppression by human chorionic gonadotropin. *Science* 184: 913 (1974).
- 374 Press, J.L.; Klinman, N.R.: Enumeration and analysis of antibody-forming precursors in the neonatal mouse. *J.Immun.* 111: 829 (1973).
- 375 Press, J.L.; Klinman, N.R.; McDevitt, H.O.: Expression of Ia antigens on hapten specific B cells. Delineation of B-cell subpopulations. *J.exp. Med.* 144: 414 (1976).
- 376 Primi, D.; Hammarstrom, L.; Moller, E.; Smith, C.I.E.: Genetic control of effector cell characteristics. *Cell.Immunol.* 47: 143 (1979).
- 377 Ptak, W.; Skowron-Cendrzak, A.: Fetal suppressor cells. Their influence on the cell-mediated immune responses. *Transplantation* 24: 45 (1977).
- 378 Ptak, W.; Zembala, M.; Gershon, R.K.: Intermediary role of macrophages in the passage of suppressor signals between T-cell subsets. *J.exp. Med.* 148: 424 (1978).
- 379 Ptak, W.; Naidorf, K.F.; Strzyzewska, J.; Gershon, R.K.: Ontogeny of cells involved in the suppressor circuit of the immune response. *Eur.J.Immunol.* 9: 495 (1979).
- 380 Ptak, W.; Naidorf, K.F.; Gershon, R.K.: Interference with the transmission of T cell-derived messages by macrophage membranes. *J.Immun.* 119: 444 (1977).
- 381 Ptak, W.; Gershon, R.K.: Immunosuppression effected by macrophage surfaces. *J.Immun.* 115: 1346 (1975).
- 382 Ptak, W.; Guninska, M.; Stachurska, B.; Czarnik, Z.: Inhibitory effect of alpha-globulins of different origin on the cell-mediated immune response in mice. *Int.Archs Allergy appl. Immun.* 53: 145 (1977).
- 383 Puck, T.T.; Waldren, C.A.; Jones, C.: Mamalian cell growth proteins. I. Growth stimulation by fetuin. *Proc.natn.Acad.Sci. USA* 59: 192 (1968).
- 384 Purtilo, D.T.; Hallgren, H.M.; Yunis, E.J.: Depressed maternal lymphocyte response to phytohaemagglutinin in human pregnancy. *Lancet* ii: 769 (1972).
- 385 Quagliata, F.; Lawrence, V.J.W.; Phillips-Quagliata, J.M.: Prostaglandin E, as a regulator of lymphocyte functions. *Cell.Immunol.* 6: 457 (1973).
- 386 Rabinovich, H.; Umiel, T.; Reisner, Y.; Sharon, N.; Globerson, A.: Characterization of embryonic liver suppressor cells by peanut agglutinin. *Cell.Immunol.* 47: 347 (1979).

- 387 Rabinowitz, S.G.: Measurement and comparison of the proliferative and antibody responses of neonatal, immature and adult murine spleen cells to T-dependent and T-independent antigens. *Cell. Immunol.* 21: 201 (1976).
- 388 Raff, M.C.; Owen, J.J.T.: Thymus derived lymphocytes: their distribution and role in the development of peripheral lymphoid tissues of the mouse. *Eur. J. Immunol.* 1: 27 (1971).
- 389 Raff, M.C.; Megson, M.; Owen, J.J.T.; Cooper, M.D.: Early production of intracellular IgM by B-lymphocyte precursors in mouse. *Nature, Lond.* 259: 224 (1976).
- 390 Raff, M.C.; Owen, J.J.T.; Cooper, M.D.; Lawton, A.R.; Megson, M.; Gathings, W.E.: Differences in susceptibility of mature and immature mouse B lymphocytes to anti-immunoglobulin induced immunoglobulin suppression *in vitro*. Possible implications for B-cell tolerance to self. *J. exp. Med.* 142: 1052 (1975).
- 391 Ramshaw, I.A.; McKenzie, I.F.C.; Bretcher, P.A.; Parish, C.R.: Discrimination of suppressor T cells of humoral and cell-mediated immunity by anti-Ly and anti-Ia sera. *Cell. Immunol.* 31: 364 (1977).
- 392 Reif, A.E.; Allen, J.M.V.: Mouse thymic iso-antigens. *Nature, Lond.* 209: 521 (1966).
- 393 Rembiesa, R.; Ptak, W.; Bubak, M.: The immunosuppressive effects of mouse placental steroids. *Experientia* 30: 82 (1974).
- 394 Rich, R.R.; Pierce, C.W.: Biological expression of lymphocyte activation. II. Generation of a population of thymus-derived suppressor lymphocytes. *J. exp. Med.* 137: 649 (1973).
- 395 Rich, S.S.; Rich, R.R.: Regulatory mechanisms in cell-mediated immune responses. I. Regulation of mixed lymphocyte reactions by alloantigen-activated thymus-derived lymphocytes. *J. exp. Med.* 140: 1588 (1974).
- 396 Rich, S.S.; Rich, R.R.: Regulatory mechanisms in cell-mediated immune responses. II. A genetically restricted suppressor of mixed lymphocyte reactions released by alloantigen-activated spleen cells. *J. exp. Med.* 142: 1391 (1975).
- 397 Robinson, J.H.; Owen, J.J.T.: Generation of T cell function in organ culture of foetal mouse thymus. *Clin. exp. Immunol.* 23: 347 (1976).
- 398 Rodriguez, G.; Andersson, G.; Wigzell, H.; Peck, A.B.: Non-T cell nature of the naturally occurring, spleen-associated suppressor cells present in the newborn mouse. *Eur. J. Immunol.* 9: 737 (1979).
- 399 Rocklin, R.E.; Kitzmiller, J.L.; Kaye, M.D.: Immuno-biology of the maternal-fetal relationship. *A. Rev. Med.* 30: 375 (1979).
- 400 Rogers, T.J.; Nowowiejski, I.; Webb, D.R.: Partial characterization of a prostaglandin-induced suppressor factor. *Cell. Immunol.* 50: 82 (1980).
- 401 Rollwagen, F.M.; Stutman, O.: Ontogeny of regulatory cells. *Fed. Proc.* 38: 2075 (1979).
- 402 Rollwagen, F.M.; Stutman, O.: Ontogeny of culture-generated suppressor cells. *J. exp. Med.* 150: 1359 (1979).
- 403 Rosenberg, Y.J.; Cunningham, A.J.: Ontogeny of the antibody-forming cells line in mice. I. Kinetics of appearance of mature B cells. *Eur. J. Immunol.* 5: 444 (1975).
- 404 Rosenstreich, D.L.: The macrophage requirement for mitogenic activation of T-lymphocytes; in Oppenheim, Rosenstreich, *Mitogens in immunobiology*, p.385 (Academic Press, New York 1975).
- 405 Rosenthal, A.S.; Shevach, E.M.: Function of macrophages in antigen recognition

- by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J.exp. Med.* 138: 1194 (1973).
- 406 Ruoslahti, E.; Engvall, E.: Immunological cross-reaction between alpha-fetoprotein and albumin. *Proc.natn.Acad.Sci.USA* 73: 4641 (1976).
- 407 Ruoslahti, E.; Terry, W.: Alpha-fetoprotein and serum albumin show sequence homology. *Nature, Lond.* 260: 804 (1976).
- 408 Ruoslahti, E.; Seppala, M.: Studies of carcino-fetal proteins. III. Development of a radioimmunoassay for alpha-fetoprotein. Demonstration of alpha-fetoprotein in serum of healthy human adults. *Int.J.Cancer* 8: 373 (1971).
- 409 Ruoslahti, E.; Pihko, H.; Seppala, M.: Alpha-fetoprotein. Immunochemical purification and chemical properties. Expression in normal state and in malignant and non-malignant liver disease. *Transplant.Rev.* 20: 38 (1974).
- 410 Ruoslahti, E.; Seppala, M.: Alpha-fetoprotein in cancer and fetal development. *Adv.Cancer Res.* 29: 275 (1979).
- 411 Sainte-Marie, G.; Leblond, C.P.: Cytological features and cellular migration in the cortex and medulla of thymus in the young adult rat. *Blood J.Hematol.* 23: 275 (1964).
- 412 Sasaki, M.; Sekezawa, T.; Abo, T.; Kunagai, K.: Heterogeneity of human T lymphocytes to bind to sheep erythrocytes and mitogenic responses to their subpopulations. *J.Immun.* 115: 1509 (1975).
- 413 Savary, C.A.; Lotzova, E.: Suppression of natural killer cytotoxicity by splenocytes from *Corynebacterium parvum*-injected, bone-marrow tolerant, and infant mice. *J.Immun.* 120: 239 (1978).
- 414 Scheid, M.P.; Hoffman, M.K.; Komuro, M.K.; Hammerling, U.; Abbott, J.; Boyse, E.A.; Cohen, G.H.; Hooper, J.A.; Schulof, R.S.; Goldstein, A.L.: Differentiation of T cells induced by preparations from thymus and by non-thymic agents. The determined state of the precursor cell. *J.exp.Med.* 138: 1027 (1973).
- 415 Scheiff, J.M.; Haumont, S.: The effect of oestradiol on thymic epithelial cells in mouse. *J.clin.Lab.Immunol.* 2: 225 (1979).
- 416 Schendel, D.J.; Bach, F.H.: H-2 and non-H-2 determinants in the genetic control of cell-mediated lympholysis. *Eur.J.Immunol.* 5: 880 (1975).
- 417 Scher, I.; Sherrow, S.O.; Wistar, R.; Asosky, R.; Paul, W.E.: B lymphocyte heterogeneity. Ontogenetic development and organ distribution of B-lymphocyte populations defined by their density of surface immunoglobulin. *J.exp. Med.* 144: 494 (1976).
- 418 Schoultz, B.; Stigbrand, T.; Tarnvik, A.: Inhibition of Pha-induced lymphocyte stimulation by the pregnancy zone protein. *FEBS Lett.* 38: 23 (1973).
- 419 Scollay, R.; Weissman, I.L.: T cell maturation. Thymocyte and thymus migrant subpopulations defined with monoclonal antibodies to the antigens Lyt-1, Lyt-2, and ThB. *J.Immun.* 124: 2841 (1980).
- 420 Scollay, R.; Kochen, M.; Butcher, E.; Weissman, I.: Lyt markers on thymus cell migrants. *Nature, Lond.* 276: 79 (1978).
- 421 Sell, S.; Sheppard, H.W., Jr.; Poler, M.: Effects of alpha-fetoprotein on murine immune responses. II. Studies on rats. *J.Immun.* 119: 98 (1977).
- 422 Senik, A.; Gresser, I.; Maury, C.; Gidlund, M.; Orn, A.; Wigzell, H.: Enhancement by interferon of natural killer cell activity in mice. *Cell.Immunol.* 44: 186 (1979).
- 423 Sheppard, H.W., Jr.; Sell, S.; Trefts, P.; Bahu, R.: Effects of alpha-fetoprotein on murine immune responses. I. Studies on mice. *J.Immun.* 119: 91 (1977).

- 424 Sherwin, W.K.; Rowlands, D.T., Jr.: Development of humoral immunity in lethally irradiated mice reconstituted with fetal liver. *J.Immun.* 113: 1353 (1974).
- 425 Sherwin, W.K.; Rowlands, D.T., Jr.: Determinants of the hierarchy of humoral immune responsiveness during ontogeny. *J.Immun.* 115: 1549 (1975).
- 426 Shustic, C.; Cohen, I.R.; Schwartz, R.S.; Latham-Griffin, E.; Waksal, S.D.: T lymphocytes with promiscuous cytotoxicity. *Nature, Lond.* 263: 699 (1976).
- 427 Sidman, C.L.; Unanue, E.R.: Development of B lymphocytes. I. Cell populations and a critical event during ontogeny. *J.Immun.* 114: 1730 (1975).
- 428 Siiteri, P.K.; Febres, F.; Clemens, L.E.; Chang, R.J.; Gondos, B.; Stites, D.: Progesterone and maintenance of pregnancy. Is progesterone nature's immunosuppressed? *Ann.N.Y.Acad.Sci.* 286: 384 (1977).
- 429 Silverman, M.S.: The macrophage in cellular and humoral immunity. *J.reticuloendoth.Soc.* 8: 105 (1970).
- 430 Silverstein, A.M.: Ontogeny of the immune response. A perspective; in Cooper, Dayton, Development of host defences, pp.1-10 (Raven Press, New York 1977).
- 431 Silverstein, A.M.; Segal, S.: The ontogeny of antigen-specific T cells. *J.exp.Med.* 142: 802 (1975).
- 432 Skowron-Andrzak, A.; Ptak, W.: Suppression of local graft-versus-host reactions by mouse fetal and newborn spleen cells. *Eur.J.Immunol.* 6: 451 (1976).
- 433 Slade, B.: Antibodies to alpha-fetoprotein cause foetal mortality in rabbits. *Nature, Lond.* 246: 493 (1973).
- 434 Slades, B.; Forrest, J.: Depression of antibody production in rats by homologous amniotic fluid. *IRCS med.Sci.* 5: 319 (1977).
- 435 Smith, C.J.P.; Kelleher, P.C.: Alpha-fetoprotein molecular heterogeneity. Physiological correlations with normal growth carcinogenesis, and tumor growth. *Biochim.biophys.Acta* 605: 1 (1980).
- 436 Smith, G.: Inhibition of cell-mediated microcytotoxicity and stimulation of mixed lymphocyte reactivity by mouse pregnancy serum. *Transplantation* 26: 278 (1978).
- 437 Smith, J.A.; Burton, R.C.; Barg, M.; Mitchell, G.F.: Maternal alloimmunisation in pregnancy. In vitro studies of T cell-dependent immunity to paternal alloantigens. *Transplantation* 25: 216 (1978).
- 438 Solomon, J.B.; Riddel, G.S.; Whyte, D.F.: Specific immunosuppressive delay of the plaque-forming response by trace amounts of antibody of maternal origin in young rats injected with mouse or sheep erythrocytes. *Immunology* 22: 219 (1972).
- 439 Sonnenfeld, G.; Mandel, A.D.; Merigan, T.C. The immunosuppressive effect of type II mouse interferon preparations on antibody production. *Cell.Immunol.* 34: 193 (1977).
- 440 Soto, A.M.; Sonnenschein, C.: Control of growth of estrogen-sensitive cells. Role for alpha-fetoprotein. *Proc.natn.Acad.Sci. USA* 77: 2084 (1980).
- 441 Soubiran, P.; Mucchielli, A.; Kerckaert, J.P.; Bayard, B.; Masseyeff, R.: Stimulatory effect of human alpha-fetoprotein and its molecular variants on *in vitro* induced lymphocyte blastogenesis. *Scand.J.Immunol.* 10: 179 (1979).
- 442 Spear, P.G.; Edelman, G.M.: Maturation of the humoral immune response in mice. *J.exp.Med.* 139: 249 (1974).
- 443 Spear, P.G.; Wang, A.-L.; Rutishauser, U.; Edelman, G.M.: Characterization of splenic lymphoid cells in fetal and newborn mice. *J.exp.Med.* 138: 557 (1973).

- 444 Spieker-Polet, H.; Polet, H.: Identification of albumin as the serum factor essential for the growth of activated human lymphocytes. *J. biol. Chem.* 251: 987 (1976).
- 445 Stanton, T. H.; Calkins, C. E.; Jandinski, J.; Schendel, D. J.; Stutman, O.; Cantor, H.; Boyse, E. A.: The Qa-1 antigenic system. Relation of Qa-1 phenotypes to lymphocyte sets, mitogen responses, and immune functions. *J. exp. Med.* 148: 963 (1978).
- 446 Steinman, R. M.; Lustig, D. S.; Cohn, Z. A.: Identification of a novel cell type in peripheral lymphoid organs of mice. III. Functional properties *in vivo*. *J. exp. Med.* 139: 1431 (1974).
- 447 Steinman, R. M.; Kaplan, G.; Witmer, M. D.; Cahn, Z. A.: Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance *in vitro*. *J. exp. Med.* 149: 1 (1979).
- 448 Steinman, R. M.; Witmer, M. D.: Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc. Natn. Acad. Sci. USA* 75: 5132 (1978).
- 449 Stern, P.; Gidlund, M.; Orn, A.; Wigzell, H.: Natural killer cells mediate efficient lysis of MHC lacking embryonal carcinoma cells. *Nature, Lond.* 283: 341 (1980).
- 450 Sterzl, J.; Silverstein, A. M.: Developmental aspects of immunity. *Adv. Immunol.* 6: 337 (1967).
- 451 Stimson, W. H.: Identification of pregnancy associated α -macroglobulin on the surface of peripheral blood leucocyte populations. *Clin. exp. Immunol.* 28: 445 (1977).
- 452 Stimson, W. H.: Studies on the immunosuppressive properties of a pregnancy-associated α -macroglobulin. *Clin. exp. Immunol.* 25: 199 (1976).
- 453 Stimson, W. H.: Are pregnancy-associated serum proteins responsible for the inhibition of lymphocyte transformation by pregnancy serum? *Clin. exp. Immunol.* 40: 157 (1980).
- 454 Stites, D. P.; Carr, M. C.; Fudenberg, H. H.: Ontogeny of cellular immunity in the human fetus. Development of responses to phytohemagglutinin and to allogeneic cells. *Cell. Immunol.* 11: 257 (1974).
- 455 Stobo, J. B.; Paul, W. F.: Differential responsiveness of T cells to phytohemagglutinin and concanavalin A as a probe for T cell subsets. *J. Immun.* 110: 362 (1973).
- 456 Stobo, J. D.; Paul, W. E.: Functional heterogeneity of murine lymphoid cells. II. Acquisition of mitogen responsiveness and of antigen during ontogeny of thymocytes and "T" lymphocytes. *Cell. Immunol.* 4: 367 (1972).
- 457 Stout, R. D.; Herzenberg, L. A.: The Fc receptor on thymus-derived lymphocytes. I. Detection of a subpopulation of murine T lymphocytes bearing the Fc receptor. *J. exp. Med.* 142: 611 (1975).
- 458 Strayer, D. S.; Kohler, H.: Immune response to phosphorylcholine. II. Nature 'auto'-anti-receptor antibody in neonatal Balb/c mice. *Cell. Immunol.* 25: 294 (1976).
- 459 Strelkauskas, A. J.; Wilson, B. S.; Dray, S.; Dodson, M.: Inversion of levels of human T and B cells in early pregnancy. *Nature, Lond.* 258: 331 (1975).
- 460 Stutman, O.; Good, R. A.: Immunocompetence of embryonic hemopoietic cells after traffic to thymus. *Transplant. Proc.* 3: 923 (1971).
- 461 Sulit, H. L.; Golub, S. H.; Irie, R. F.; Gupta, R. K.; Grooms, G. A.; Morton, L. D.: Human tumor cells grown in fetal calf serum and human serum. Influences on the tests for lymphocyte cytotoxicity, serum blocking and serum arming effects. *Int. J. Cancer* 17: 461 (1976).

- 462 Sullivan, A.K.; Jerry, L.M.; Rowden, G.; Lewis, M.G.; Pitzele, R.; Law, T.; Adams, L.S.; Le Thi, H.; Shea, M.: Fetal antigens on the surface of human lymphoid cells. *J.exp.Med.* 143: 1557 (1976).
- 463 Suzuki, K.; Tomasi, T.B., Jr.: Immune responses during pregnancy. Evidence of suppressor cells for splenic antibody response. *J.exp.Med.* 150: 898 (1979).
- 464 Sy, M.S.; Dietz, M.H.; Germain, R.N.; Benacerraf, B.; Greene, M.I.: Antigen and receptor driven regulatory mechanisms. IV. Idiotype-bearing I-J⁺ suppressor T cell factors induce second order suppressor T cells which express anti-idiotypic receptors. *J.exp.Med.* 151: 1183 (1980).
- 465 Tada, T.; Taniguchi, M.; David, C.S.: Properties of the antigen-specific suppressive T cell factor in the regulation of antibody response of the mouse. IV. Special sub-region assignment of the gene(s) that code for the suppressive T cell factor in the H-2 histocompatibility complex. *J.exp.Med.* 144: 713 (1976).
- 466 Tada, T.; Takemori, T.; Okumura, K.; Nonaka, M.; Tokushisa, T.: Two distinct types of helper T cells involved in the secondary antibody response. Independent and synergistic effects of Ia⁻ and Ia⁺ helper T cells. *J.exp.Med.* 147: 446 (1978).
- 467 Tada, T.; Taniguchi, M.; Takemori, T.: Products of primed suppressor T cells and their products. *Transplant. Rev.* 26: 106 (1975).
- 468 Tada, T.; Okumura, K.: The role of antigen-specific T cell factors in the immune response. *Adv.Immunol.* 28: 1 (1979).
- 469 Tadakuma, T.; Pierce, C.W.: Mode of action of a soluble immune response suppressor (SIRS) produced by concanavalin A-activated spleen cells. *J.Immun.* 120: 481 (1978).
- 470 Takeya, K.; Nomoto, K.: Characteristics of antibody response in young or thymectomized mice. *J.Immun.* 99: 831 (1967).
- 471 Than, G.N.; Csaba, I.F.; Karg, N.J.; Szabo, D.G.; Ambrus, M.; Bajtai, G.: Immunosuppressive effect of pregnancy-associated alpha-glycoprotein. *Lancet ii*: 515 (1975).
- 472 Theze, J.; Waltenbaugh, C.; Dorf, M.E.; Benacerraf, B.: Immunosuppressive factor(s) specific for L-glutamic acid³⁰-L-tyrosine³⁰ (GT). II. Presence of I-J determinants on the GT-suppressive factor. *J.exp.Med.* 146: 287 (1977).
- 473 Thong, Y.H.; Steele, R.W.; Vincent, M.M.; Hensen, S.A.; Bellanti, J.A.: Impaired *in vitro* cell-mediated immunity to rubella virus during pregnancy. *New Engl.J.Med.* 289: 604 (1973).
- 474 Toder, V.; Blank, M.; Nebel, L.: Immunosuppressive effect of alpha-fetoprotein at different stages of pregnancy in mice. *Proc. 12th Int. Leucocyte Culture Conf.*, 1978.
- 475 Tomasi, T.B.; Murgita, R.A.; Thompson, R.L.; Calvanico, N.J.; Dattwyler, R.J.: Alpha-fetoprotein and the immune response during murine pregnancy and neonatal development; in Cooper, Dayton, Development of host defences, p.273 (Raven Press, New York 1977).
- 476 Tormey, D.C.; Mueller, G.C.: Biological effects of transferrin on human lymphocytes *in vitro*. *Expl. Cell Res.* 74: 220 (1972).
- 477 Tormey, D.C.; Imrie, R.C.; Mueller, G.C.: Identification of transferrin as a lymphocyte growth promoter in human serum. *Expl. Cell Res.* 74: 163 (1972).
- 478 Tyan, M.L.: Immunosuppressive properties of mouse amniotic fluid. *Proc.Soc. exp.Biol.* 151: 343 (1976).

- 479 Umiel, T.; Auerbach, R.: Studies on the development of immunity. The graft-versus-host reaction. *Pathol. 7*: 27 (1973).
- 480 Umiel, T.; Globerson, A.; Trainin, N.: Development of immunosuppressor cells. Exertion of specific suppression by neonatal liver cells. *Transplantation 24*: 282 (1977).
- 481 Unanue, E.R.: The regulatory role of macrophages in antigenic stimulation. *Adv. Immunol. 15*: 95 (1972).
- 482 Uriel, J.; Bouillon, D.: Affinity chromatography of human, rat, and mouse alpha-fetoprotein on estradiol sepharose absorbants. *FEBS Lett. 53*: 305 (1975).
- 483 Uriel, J.; Bouillon, D.; Aussel, C.; Dupiers, M.: Alpha-fetoprotein. The major high-affinity estrogen binder in rat uterine cytosols. *Proc.natn.Acad.Sci. USA 73*: 1452 (1976).
- 484 Viet, B.; Michael, J.G.: Characterization of an immunosuppressive factor present in mouse serum. *J.Immun. 111*: 341 (1973).
- 485 Vitetta, E.S.; Melcher, U.; McWilliams, M.; Lamm, M.E.; Phillips-Quagliata, J.; Uhr, J.W.: Cell surface immunoglobulin. XI. The appearance of an IgD-like molecule on murine lymphoid cells during ontogeny. *J.exp.Med. 141*: 206 (1975).
- 486 Vitetta, E.S.; Cambier, J.; Spiva, D.; Kettman, J.R.; Uhr, J.W.: Immunoglobulins on murine B lymphocytes: Implications for differentiation, stimulation, and induction of tolerance; in Cooper, Dayton, Development of host defenses, p.75 (Raven Press, New York 1977).
- 487 Vitetta, E.S.; Baur, S.; Uhr, J.W.: Cell surface immunoglobulin. II. Isolation and characterization of immunoglobulin from mouse splenic lymphocytes. *J.exp.Med. 134*: 242 (1971).
- 488 Waksman, B.H.; Namba, Y.: On soluble mediators of immunologic regulation. *Cell.Immunol. 21*: 161 (1976).
- 489 Watanabe, N.; Kojima, S.; Ovary, Z.: Suppression of IgE antibody production in SJL mice. I. Nonspecific suppressor T cells. *J.exp.Med. 143*: 833 (1976).
- 490 Watanabe, N.; Kojima, S.; Shen, F.W.; Ovary, Z.: Suppression of IgE antibody production in SJL mice. II. Expression of Ly-1 antigen on helper and nonspecific suppressor T cells. *J.Immun. 118*: 485 (1977).
- 491 Weiss, A.: Suppression of adult rat lymphoid proliferative responses by homologous neonatal serum. *J.Immun. 118*: 1121 (1977).
- 492 Weissman, I.L.: T cell maturation and the ontogeny of splenic lymphoid architecture; in Battisto, Streilen, Immuno-aspects of the spleen, p.77 (Elsevier/North Holland Biomedical Press, Amsterdam 1977).
- 493 Wigzell, H.: Specific affinity fractionation of lymphocytes using glass or plastic bead columns. *Scand.J.Immunol., suppl. 5*, p.23 (1976).
- 494 Wigzell, H.; Murgita, R.A.; Peck, A.B.; Binz, H.; Frischnecht, H.; Peterson, P.; Sege, K.: Induction and inhibition of lymphocyte functions. A note on complexity and consequences; in Janicki, Escape from immune surveillance. An interpretive survey of the interface between immune mechanisms and disease, p.13 (Academic Press, New York 1979).
- 495 Williams, R.C.; Korsmeyer, S.J.: Studies of human lymphocyte interactions with emphasis on soluble suppressor activity. *Clin.Immunol.Immunopathol. 9*: 335 (1978).
- 496 Wolf, R.L.; Lomnitzer, R.; Rabson, A.R.: An inhibitor of lymphocyte prolifera-

- tion and lymphokine production released by unstimulated foetal monocytes. *Clin. exp. Immunol.* 27: 464 (1977).
- 497 Wu, A.M.; Till, J.E.; Siminovitch, McCulloch, E.A.: A cytological study of the capacity for differentiation of normal hematopoietic colony-forming cells. *J. Cell. Physiol.* 69: 177 (1967).
- 498 Wu, S.; Bach, F.H.; Averbach, R.: Cell-mediated immunity: Differential maturation of mixed leukocyte reaction and cell-mediated lympholysis. *J. exp. Med.* 142: 1301 (1975).
- 499 Wyle, F.A.; Kent, J.R.: Immunosuppression by sex steroid hormones. I. The effect upon PHA- and PPD-stimulated lymphocytes. *Clin. exp. Immunol.* 27: 407 (1977).
- 500 Yachnin, S.: Fetuin, an inhibitor of lymphocyte transformation. The interaction of fetuin with phytoimitogens and a possible role for fetuin in fetal development. *J. exp. Med.* 141: 242 (1975).
- 501 Yachnin, S.; Lester, E.: Inhibition of human lymphocyte transformation by human alpha-fetoprotein (HAFP); comparison of foetal and hepatoma HAFP and kinetic studies of *in vitro* immunosuppression. *Clin. exp. Immunol.* 26: 484 (1976).
- 502 Youtananukorn, V.; Matangkasombut, P.: Specific plasma factors blocking human maternal cell-mediated immune reaction to placental antigens. *Nature new Biol.* 242: 110 (1973).
- 503 Zambala, M.; Asherson, G.L.; Noworolski, J.; Mayhew, B.: Contact sensitivity to picryl chloride. The occurrence of B suppressor cells in the lymph nodes and spleen of immunized mice. *Cell. Immunol.* 25: 266 (1976).
- 504 Zambala, M.; Asherson, G.L.; Mayhew, B.; Krejci, J.: In vitro absorption and molecular weight of specific T cell suppressor factor. *Nature, Lond.* 253: 72 (1975).
- 505 Zielske, J.U.; Golub, S.H.: Fetal calf serum-induced blastogenic and cytotoxic responses of human lymphocytes. *Cancer Res.* 36: 3842 (1976).
- 506 Zimmerman, E.F.; Madappally, M.M.: Sialyltransferase. Regulation of alpha-fetoprotein microheterogeneity during development. *Biochem. J.* 134: 807 (1973).
- 507 Zimmerman, E.F.; Voorting-Hawking, M.; Michael, J.G.: Immunosuppression by mouse sialylated alpha-fetoprotein. *Nature, Lond.* 265: 354 (1977).

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